University of Veterinary Medicine Hannover

Clinic for Poultry

Interference of infectious bursal disease virus with the development of the gut-associated immune system and the establishment of the gut microbiota

THESIS

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY
(PhD)

awarded by the University of Veterinary Medicine Hannover

by

Li Li

(Sichuan, China)

Hannover 2017

Supervisor:	Prof. Dr. Silke Rautenschlein, PhD
Supervision Group:	Prof. Dr. Silke Rautenschlein, PhD Prof. Dr. Georg Herrler Prof. Dr. Reimar Johne
1st Evaluation:	Prof. Dr. Silke Rautenschlein, PhD (Clinic for Poultry, University of Veterinary Medicine Hannover, Germany) Prof. Dr. Georg Herrler (Institute of Virology, University of Veterinary Medicine Hannover, Germany) Prof. Dr. Reimar Johne (German Federal Instutue for Risk Assessment, Berlin, Germany)
1st Evaluation:	Prof. Dr. Michael Hess (Vetmed Universität Wien, Dept. of Farm Animal and Veterinary, Puclic health Clinic for Poultry and Fish, Medicine Veterinärplatz 1 1210 Wien)
Date of final exam:	30.11.2017

Li Li was supported by the Chinese Scholarship Council.

To my family

Publications:

L. Li, C. Pielsticker, Z. Han, T. Kubasová, I Rychlik, B. Kaspers, S. Rautenschlein. Infectious bursal disease virus inoculation modifies *Campylobacter jejuni*-host interaction in broilers. Gut Patho 10(1): 13.

L. Li, T. Kubasová, I Rychlik, Frederic J. Hoerr, S. Rautenschlein. Infectious bursal disease virus infection leads to changes in the gut associated-lymphoid tissue and the microbiota composition. PloS one 13(2): e0192066.

Han Z, **Willer T, Li L, Pielsticker C, Rychlik I, Velge P, Kaspers B, Rautenschlein** S. The influence of the gut microbiota composition on *Campylobacter jejuni* colonization in chickens. Infect Immun. 2017 Aug 14. pii: IAI.00380-17. doi: 10.1128/IAI.00380-17. [Epub ahead of print]

Oral presentations:

10/09/2016 XVIth Avian Immunol Reasearch Group (AIRG) meeting 2016, münchen, Germany

Infectious bursal disease virus infection modifies coloniyation pattern and immune responses following *Campylobacter jejuni* inoculation of broilers
L. Li, C. Pielsticker, Z. Han, T. Willer, I. Rychlik, S. Rautenschlein.

Poster presentations:

04/09/2017 XXth World Veterinary Poultry Association (WVPA) Congress. Edinburgh, UK.

Infectious bursal disease virus modifies *Campylobacter jejuni*-host interaction in broilers: influence of timing between subsequent infections
L. Li, C. Pielsticker, Z. Han, T. Willer, I. Rychlik, S. Rautenschlein.

Table of Contents

List of abbreviations

°C	degree celsius
µg	microgram
µl	microliter
AA	amino acid
aIBDV	attenuated strain of infectious bursal disease virus
AIV	Avian influenza virus
BF	bursa of Fabricius
BMDCs	bone marrow derived dendritic cells
Bp	base pairs
C. jejuni	*Campylobacter jejuni*
CAV	chicken Anemia virus
CCR	chemokine receptor
CEC	chicken embryo cell
CEB	chicken embryo bursa
CEF	chicken embryo fibroblasts
CFU	colony forming units
ChIFN	chicken interferon
cIBDV	classical infectious bursal disease virus
CMI	cell mediated immunity
CO_2	carbon dioxide
COX	cyclooxygenase
CT	caecal tonsils
CXCLi	chemokine (C-X-C Motif) ligand
DC	dendritic cell
DNA	deoxyribonucleic acid
dph	days post hatch
dpi	days post infection
ds	double-stranded
DXV	Drosophila X virus
ED	Embryonation day
ELISA	enzyme-linked immunosorbent assay
GALT	gut associated lymphoid tissue
GILZ	glucocorticoid-induced leucine zipper protein
GIT	gastrointestinal tract
hpi	hours post infection (inoculation)
HSP	heat shock protein
HW	higher weight
IBD	infectious bursal disease
IBDV	infectious bursal disease virus
IBDV-B2	IBDV Bursine-2 strain
IBDV-D78	intermediate IBDV vaccine strain D78

IBV	infectious bronchitis virus
ICX	IBD immune complex
IEC	intestinal epithelial cell
IEL	intraepithelial lymphocytes
IFA	immunofluorescence Assay
IFN	interferon
Ig	immunoglobulin
IL	interleukin
ILTV	infectious laryngo tracheitis virus
IM-IBDV	virulent IBDV
iNOS	inducible nitric oxide synthase
IPNV	infectious pancreatic necrosis virus
LOS	lipo-oligosaccharides
LP	lamina propria
LPL	LP lymphocyte
LT	Layer type
LW	low weight
M cells	microfold cells
MDA	maternally derived-antibodies
MABs	monoclonal antibodies
MALT	mucosa associated lymphoid tissue
mCCDA	modified charcoal cefoperazone deoxycholate agar
MCFA	medium-chain fatty acid
MD	Marek's disease
MDV	Marek's disease virus
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
ND	Newcastle disease
NDV	Newcastle disease virus
NK	natural killer
NS	nonstructural
O2	oxygen
OD	optical density
ORF	open reading frame
OTU	operational taxonomic unit
OV	oyster virus
PAMPS	pathogen-associated molecular patterns
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PKR	protein kinase R

PP	peyer’s patches
RdRP	RNA-dependent RNA polymerase
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
S/P	sample to positive ratio
SCFA	short-chain fatty acid
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SM	spleen macrophage
SPF	specific pathogen free
ssp.	subspecies
ssRNA	single stranded RNA
$TCID_{50}$	50% tissue culture infective dose
TCR	T cell receptor
TGF	transforming growth factor
TLR	Toll-like receptor
TNF	tumor necrosis factor
TNFSF	tumor necrosis factor super family
TV	tellina virus
vIBDV	virulent IBDV
VNT	virus neutralization test
VP	viral protein
VP1-VP4	viral protein1-4
VP2	viral protein2
VPg	viral protein genome-linked
vvIBDV	very virulent IBDV
WBES	wheat-based diet with non-starch polysaccharides-degrading enzyme supplementation

List of Figures

List of Tables

1. Summary

Li Li

Interference of infectious bursal disease virus with the development of the gut-associated immune system and the establishment of the gut microbiota

Infectious bursal disease virus (IBDV) is an immunosuppressive virus of young chickens, which may lead to high morbidity and mortality rates in susceptible birds. The immunosuppressive nature allows secondary pathogens to invade the host, which subsequently may exacerbate the disease and lead to economic losses. IBDV-pathogenesis studies have focused mainly on primary lymphoid organs. However, neither the effect of very virulent (vv) IBDV infection on gut associated lymphiod tissues (GALT) nor the possible correlation to the gut microbiota composition has been investigated so far.

The first aim of this thesis was to investigate the effect of vvIBDV on the (GALT) as well as on the gut microbiota composition. Different immune parameters of the GALT in the bursa of Fabricious (BF), caecal tonsil (CT) and caecum were examined more closely. The gut microbiota composition was determined in the caecal content because the caecum harbors a more diverse microbial community compared to other intestinal sections and it is physically associated with the CT. Broiler chickens were inoculated with vvIBDV at 15 or 14 days post hatch (dph), when the maternally derived antibodies (MDA) were below the breakthrough titer of the virus. Viral-antigen-positive cells were observed in the BF, CT and caecum. vvIBDV-inoculated birds showed a significantly higher number of CD4+ and CD8ß+ lamina propria lymphocytes (LPL) and a decrease in the number of B cells in the BF, CT and caecum compared to virus-free controls ($P < 0.05$). Furthermore, vvIBDV infection also led to a decrease in the number of mast cells, IgA+ as well as CD4+ and CD8ß+ intraepithelial lymphocytes (IEL) in the caecum in comparison to virus-free controls. vvIBDV infection caused a modulation of the gut microbiota composition in the caecal content. This study clearly confirmed an immunosuppressive effect of vvIBDV on the GALT and for the first time a modulatory effect of vvIBDV on the microbiota was demonstrated. These changes

might allow pathogens to colonize IBDV-infected chickens and eventually to overcome the muco-intestinal-barrier.

The aim of study 2 was to investigate the effects of vvIBDV on potential, secondary pathogens in the gut. We selected *Campylobacter jejuni* (*C. jejuni*) to follow up on this approach because it is considered as a commensal in healthy birds and recent studies indicate that it may induce lesions and lead to disease. Broiler chickens were inoculated with vvIBDV at 14 days post hatch, when the MDA were below the break through level of the virus. At seven (experiment A) or nine (experiment B) days post vvIBDV infection, the birds were inoculated with *C. jejuni*. The *C. jejuni*-colonization pattern was comparable between mono-inoculated groups of both experiments, but it varied for vvIBDV + *C. jejuni* co-inoculated groups. In experiment A significantly higher numbers of colony forming units (CFU) of *C. jejuni* were detected in caecum of co-inoculated compared to *C. jejuni*-mono-inoculated birds in the early phase post bacterial inoculation (pbi). In experiment B the clearance phase was affected in the co-inoculated group with significantly higher CFU at 21 days pbi ($P < 0.05$). vvIBDV-infection led to a depression in lamina propria B-cell numbers, total bursal IgA-mRNA expression and the anti-*C. jejuni* antibody response starting at 14 days pbi. In addition, both pathogens affected the microbiota composition.

Overall, this research demonstrates that vvIBDV infection had a significant impact on GALT and led to a modulation of the gut microbiota composition. vvIBDV infection led to an systemtic and local immunosuppression, which affected the colonization pattern of *C. jejuni*. We speculate that the humoral immunity might play an important role especially during the *C. jejuni* clearance phase. The results of this study not only suggest a possible link or interaction between IBDV-infection and the development of the gut-associated immune system, but also with the gut microbiota. Understanding the mechanism of *C. jejuni* infection in poultry is critical for eliminating the risk to public health. Due to the fact that broiler chickens are the main source of food-borne *C. jejuni* infection in humans, we suggest that a stronger surveillance of immunosuppressive pathogens, such as IBDV in chickens, might be advantageous for a better control of *Campylobacter* infections in consumers.

2. Zusammenfassung

Li Li

Einfluss des Infektiösen Bursitis Virus auf die Entwicklung des Darm-assoziierten Immunsystems und die Darmmikroflora

Das Infektiöse Bursitis Virus (IBDV) ist ein immunsuppressives Virus junger Hühner, welches zu hohen Morbiditäts-und Mortalitätsraten in empfänglichen Tieren führen kann. Aufgrund der immunsuppressiven Eigenschaften des Virus können Sekundärerreger in den Wirt eindringen, was darauf folgend zu einer Verschlechterung der Erkrankung und zu wirtschaftlichen Verlusten führen kann. Untersuchungen zur IBDV-Pathogenese konzentrierten sich hauptsächlich auf die primären Lymphorgane. Jedoch wurden bis jetzt weder der Einfluss des hochvirulenten (vv) IBD Virus auf das Darm-assoziierte lymphatische Gewebe (gut-associated lymphoid tissue, GALT) noch auf die Zusammensetzung der Darmflora untersucht. Der erste Arbeitsansatz der These war, den Einfluss des vvIBDV auf das GALT und die Zusammensetzung der Darmmikroflora zu untersuchen. Unterschiedliche Immunparameter des GALT unter Einschluss der Bursa Fabricii (BF), Zäkaltonsillen (ZT) und Zäkum wurden näher betrachtet. Die Zusammensetzung der Darmmikroflora wurde im Zäkuminhalt bestimmt, da das Zäkum im Vergleich zu anderen Darmabschnitten eine vielfältigere, mikrobielle Flora besitzt und anatomisch mit den Zäkaltonsillen zusammenhängt. Am 15. (Versuch 1) oder 14. Tag (Versuch 2) nach dem Schlupf wurden Broiler mit vvIBDV inokuliert, als die maternalen Antikörper unter dem Durchbruchtiter des Virus lagen. Virus-Antigen-positive Zellen wurden in der BF, in den ZT und im Zäkum detektiert. VvIBDV-inokulierte Hühner zeigten eine signifikant höhere Anzahl an CD4+ und CD8ß+ Lymphozyten in der Lamina Propria (LPL) und eine Reduktion der Anzahl an B-Zellen in der BF, ZT und Zäkum im Vergleich zu Virus-freien Kontroll-Tieren ($P < 0.05$). Außerdem führte die IBDV Infektion zur Reduktion der Anzahl an Mastzellen, IgA+ und auch CD4+ und CD8ß+ intraepithelialen Lymphozyten (IEL) im Zäkum im Vergleich zu Virus-freien Kontroll-Tieren. Die vvIBDV Infektion führte zu einer Veränderung der Zusammensetzung der Darmmikroflora im Zäkuminhalt. Diese Studie bestätigt den

immunsuppressiven Effekt des vvIBDV auf das GALT, und zum ersten Mal wurde ein modulatorischer Effekt des vvIBD Virus auf die Darmmikroflora gezeigt. Diese Veränderungen könnten Pathogenen ermöglichen, IBDV-infizierte Hühner zu besiedeln und schließlich die muko-intestinale Barriere zu überwinden.

Der Arbeitsansatz der Studie 2 war, die Auswirkungen des vvIBDV auf potentielle, sekundäre Pathogene im Darm zu untersuchen. Wir wählten *Campylobacter jejuni* (*C. jejuni*) aus, um dieser Frage auf den Grund zu gehen, da *C. jejuni* in gesunden Vögeln als Kommensale gilt und Studien gezeigt haben, dass er Läsionen induzieren und zu einer Erkrankung führen kann. Am 14. Tag nach dem Schlupf wurden Broiler mit vvIBDV infiziert, als die maternalen Antikörper unter dem Durchbruchtiter des Virus lagen. An 7 (Experiment A) oder 9 (Experiment B) Tagen nach der IBDV Infektion wurden die Tiere mit *C. jejuni* inokuliert. Das *C. jejuni* Kolonisationsmuster zwischen mono-infizierten Gruppen beider Experimente war vergleichbar, aber es unterschied sich zwischen vvIBDV und *C. jejuni* ko-inokulierten Gruppen. In Experiment A wurde eine signifikant höhere Zahl Kolonie-bildender Einheiten (CFU) *C. jejuni* im Zäkum ko-inokulierter Tiere im Vergleich zu mono-inokulierten Tieren in der frühen Phase nach bakterieller Infektion gefunden. In Experiment B wurde die Ausscheidungsphase in der ko-inokulierten Gruppe mit signifikant höheren CFU am 21. Tag nach bakterieller Infektion beeinflusst. Die vvIBDV Infektion führte zu einem Rückgang an B Zellen in der Lamina Propria, der Expression von IgA-mRNA und der anti-*C. jejuni* Antikörperantwort ab dem 14. Tag nach bakterieller Infektion. Außerdem beeinflussten beide Pathogene die Zusammensetzung der Darmmikroflora.

Insgesamt zeigen diese Untersuchungen, dass vvIBDV einen signifikanten Einfluss auf das darm-assoziierte lymphatische Gewebe hatte und zu Veränderungen der Zusammensetzung der Darmmikroflora führte. vvIBDV induzierte eine systemische und lokale Immunsuppression, welche das Kolonisationsmuster von *C. jejuni* beeinflusste. Es kann spekuliert werden, dass die humorale Immunität eine wichtige Rolle vor allem während der Kontrolle der späten Ausscheidungsphase von *C. jejuni* spielt. Die Ergebnisse dieser Studie zeigen nicht nur einen möglichen Zusammenhang oder ein mögliches Zusammenspiel zwischen einer IBDV-Infektion und der Entwicklung des GALT, sondern auch mit der Darmmikroflora. Die Pathogenese einer *C. jejuni* Infektion beim Geflügel besser zu verstehen, ist von entscheidender Bedeutung, um Kontrollmaßnahmen zu verbessern und

damit das öffentliche Gesundheitsrisiko zu reduzieren. Da Broiler eine der Haupteintragsquellen für eine lebensmittelbedingte *C. jejuni* Infektion beim Menschen darstellen, gehen wir davon aus, dass eine stärkere Überwachung immunsuppressiver Erreger, wie beispielsweise IBDV bei Hühnern, nützlich in Hinblick auf eine bessere Kontrolle von *Campylobacter* Infektionen bei Verbrauchern sein kann.

3. Introduction

The virus associated with infectious bursal disease (IBD) induces an immunosuppressive condition in young chickens, which may lead to high morbidity and mortality rates in susceptible birds. Immature IgM+ B-lymphocytes are the target cells for IBDV. During infection, both humoral and cellular immune responses are affected due to depletion of IgM+ B-lymphocyte precursors, as well as suppression of macrophage function. IBDV-pathogenesis studies have focused mainly on primary lymphoid organs, little is known, however, about the effects of IBDV on the gut-associated immune system. Limited studies have demonstrated the effect of both innate and adaptive immune cells on gut microbiota composition in chickens, but it has been shown that CD45-, Rag-and CD45Rag-deficient mice had different gut microbiota composition in comparison to wild-type (WT) mice (Dimitriu et al., 2013). This suggested that microbiota composition might be influenced by the development of the specific mucosal immune system (Kosiewicz et al., 2014). IBDV may replicate in lymphoid cells and macrophages of the gut associated lymphoid tissue (GALT) such as the bursa of Fabricius (BF) and caecal tonsils (CT). Limited studies indicated that IBDV might induce mucosal lesions in the gut, with an increase in mucus as well as a decrease in the number of mast cells. However, it is not known whether early IBDV infection modifies the development of local immune cells in the gut, thereby having an indirect effect on the evolving microbiota composition. It was suggested that IBDV-induced immunosuppression in the early phase of the chicken's growing period may lead to subsequent problems with secondary infections, including gut-associated diseases. Recent experimental studies demonstrated increased susceptibility for *Campylobacter jejuni* (*C. jejuni*) colonization. Increased *C. jejuni* colonization and shedding rates were observed in IBDV-vaccinated chickens, more severe lesion development was observed in birds that were coinfected with *C. jejuni* and IBDV compared to IBDV mono-inoculated birds. Therefore, coinfection of IBDV with *C. jejuni* has, from the perspective of food safety, a significant impact on poultry production. However, the interactions between the pathogens and the host are not known.

The goal of the two present studies was to understand more about the effect of IBDV on the gut-associated immune system, and subsequently on gut microbiota composition. In study 1,

we investigated the effects of IBDV infection on the development of the gut-associated immune system and gut microbiota composition in the caecum. Based on the results, we conducted study 2 to investigate the effects of IBDV-induced immunosuppression on *C. jejuni* colonization pattern and pathogenesis.

We expect that these studies will help to better understand the effect of IBDV-induced immunosuppression on the development of gut-associated lymphoid tissues (GALT) as well as the effect of IBDV on gut microbiota composition. It will help to understand this complex and dynamic interaction and possibly allows for improvement upon current prophylactic strategies against IBD as well as gut-associated diseases.

4. Literature review

4.1. Infectious bursal disease virus

4.1.1. History

Infectious bursal disease virus (IBDV) is the causative agent of infectious bursal disease (IBD). The virus type was identified in the 1970s, following the first reported cases in the United States in 1957 (Cosgrove, 1962). It was isolated from broiler chickens in Gumboro, Delaware, USA and is therefore also known as Gumboro disease. It is an acute and highly contagious disease in young chickens (Vasconcelos and Lam, 1995). The most IBDV-susceptible phase in chicken development is between three and six weeks of age (Vasconcelos and Lam, 1995). From 1960 to 1964, this disease spread to most areas in the USA (Lasher and Davis, 1997). In the late 60s and beginning of the 1970s, it spread to Europe and the Middle East, southern and western Africa, India, the Far East, and Australia (Faragher et al., 1974; Firth, 1974; van den Berg et al., 2000). To date, this virus prevails in most of the poultry-producing regions of the world and contributes to great economic losses in the poultry industry (Qi et al., 2014; Rehman et al., 2016). The virulence, infective dose, virus strain, age of the birds, genetic susceptibility of the breed, route of infection, presence or absence of neutralizing antibodies, and environmental conditions, as well as management practices, play a role as influencing factors on the economic impact of the disease (Aricibasi et al., 2010; Berg, 2000; Brandt et al., 2001; Rautenschlein et al., 2007; Tippenhauer et al., 2013).

4.1.2. *Etiology*

IBDV is a non-enveloped, bisegmented, double-stranded (ds) RNA virus belonging to the *Birnaviridae* family (Dobos, 1976; Dobos, 1979b; Müller et al., 1979a) and represents the prototype member of the genus *Avibirnavirus* (Leong et al., 2000). Other family members can be found in fish, crustaceans (infectious pancreatic necrosis virus, IPNV), insects (Drosophila X virus, DXV), and bivalve molluscs (tellina virus,TV, oyster virus, OV and crab virus, CV) (Leong et al., 2000). IBDV is highly contagious and may lead to high morbidity and mortality rates in infected birds. The most important characteristic of this disease is

immunosuppression; due to losses associated with secondary infections, it is considered as one of the major economic issues in the poultry industry worldwide.

4.1.3. *Structure of the virus*

IBDV is a single-shelled, non-enveloped virus with a diameter varying from 55-60 nm and a buoyant density of 1.31-1.34 g/ml in CsCl (Jungmann et al., 2001). The capsid of the virion is composed of a single layer of 32 capsomeres, arranged in a 5:3:2 symmetry (Müller et al., 2003). Figure 1 shows the basic structure of IBDV. IBDV contains two dsRNA segments, which have been designated as A and B. Segment A, which measures about 3254 base pairs (bp) in length, consists of two partly overlapping open reading frames (ORFs): the smaller ORF encodes viral protein (VP) VP5, a short 17-kDa non-structural protein (Berg, 2000a; Chevalier et al., 2004; Kibenge et al., 1988; Kibenge et al., 1990; Vakharia et al., 1994), the larger ORF encodes a precursor polyprotein (N-VPX-VP4-VP3-C) (Chevalier et al., 2002). Three proteins are obtained after autocatalytic cleavage: two structural proteins, VP2 (48k-Da) and VP3 (32k-Da); and another non-structural protein called serine protease VP4 (28 k-Da) (Chevalier et al., 2002). The smaller genome segment B (2817 bp) encodes VP1 (Ursula et al., 2004).

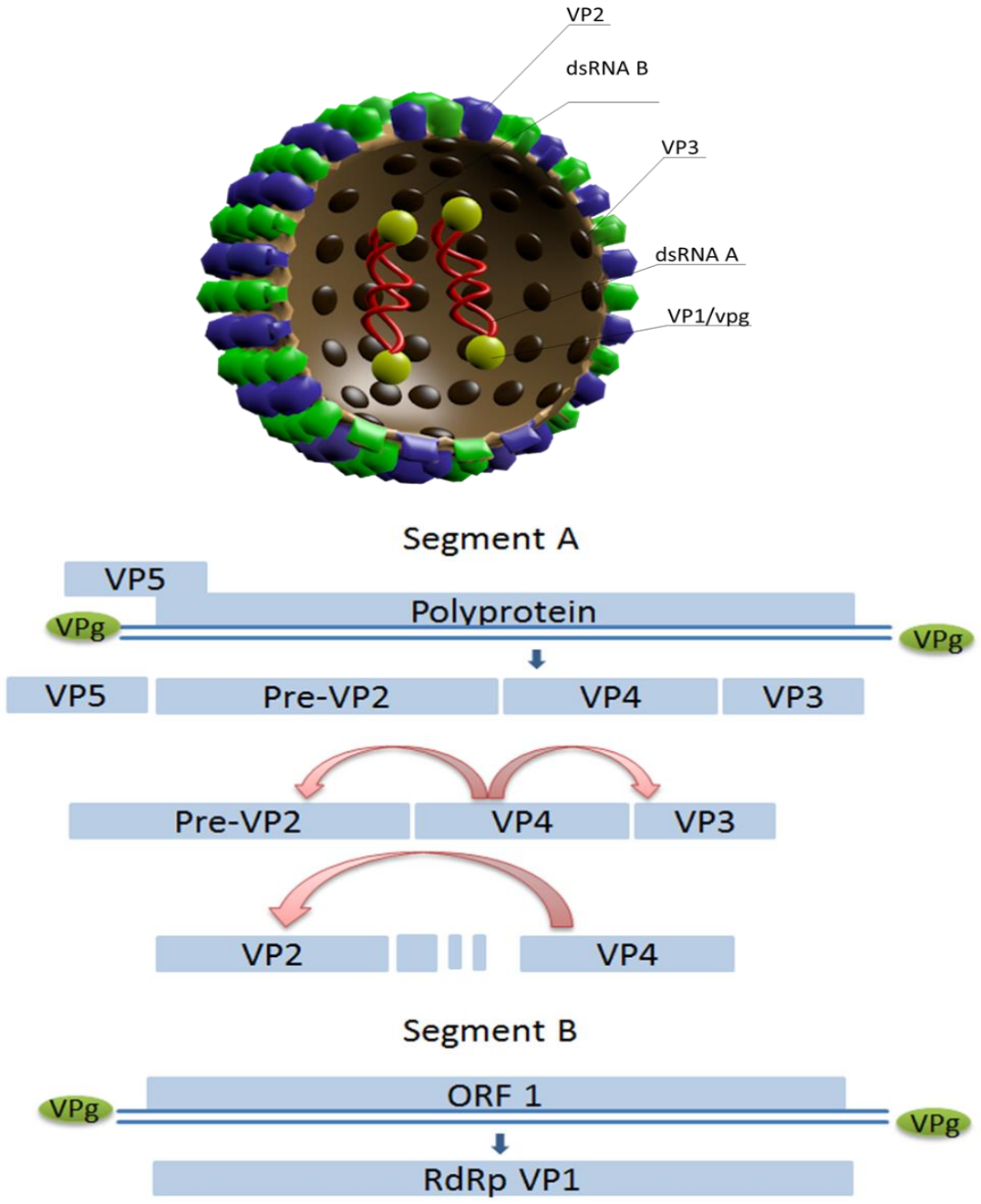

Figure 1: Morphology and structure of the IBDV genome. Two segments (A, B) encode for five proteins. The whole genome size is about 6 kb (Rehman et al., 2016). Figure is adapted from viralzone.

RdRp: RNA-dependent RNA polymerase

ORF: Open reading frame

VPg: Viral protein genome-linked

VP: Viral protein

4.1.4. *The function of viral proteins in IBDV*

Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has identified five proteins in IBDV: VP1, VP2, VP3, VP4 and VP5 (Dobos, 1979a; Hudson et al., 1986). Table 1 provides a summary of the protein functions. VP1 (a 97-k-Da protein) is a RNA-dependent RNA polymerase protein (RdRp) and is present as a free polypeptide and as a genome-linked protein in the virion (Kibenge and Dhama, 1997; Müller and Nitschke, 1987). It is covalently linked to the 5' ends of both genomic RNA segments via serine-5´-GMP phosphodiester bonds and exhibits an organization similar to other viral RdRps (von Einem et al., 2004). VP1 has been considered multi-functional and plays an important role in the encapsidation of viral particles (Lombardo et al., 1999), the replication of the genome, and the synthesis of mRNA (Lombardo et al., 1999). It has been shown that VP1 is able to contribute to the virulence of IBDV (Liu and Vakharia, 2004).

VP2 has been widely studied. It is a highly hydrophobic and conformation dependent protein (Müller et al., 1992). It was demonstrated that this protein is the only component of the icosahedral capsid (Xu et al., 2011). VP2 is considered to be the major antigen that elicits a host-protective immune response, which was demonstrated by the fact that all neutralizing monoclonal antibodies (MAB) react in immune precipitation assays (Müller et al., 1992; Van den Berg et al., 1996). Expression/deletion studies have shown that IBDV has a hyper-variable region which is located in the 206 amino acid (aa) to 350 aa area of the VP2 gene (Xu et al., 2011). This represents a major conformational, neutralizing antigenic domain responsible for cell antigenic and pathogenic variation (Xu et al., 2011). In addition to its contribution to virulence, this protein modulates cell tropism (Azad et al., 1985; Becht et al., 1988a; Castón et al., 2001; Fahey et al., 1989). There are three distinct domains in this protein, including the bare, shell, and projection domain. Bare and shell are formed by the conserved N-and C-terminal stretches of VP2 (Müller et al., 1992; Van den Berg et al., 1996). Within the projection, there are two loops (PDE and PFG). The loops regulate the sealing of the interior and of the projection domain (Lin et al., 2007). Moreover, they play a significant role in the infectivity of cell cultures and in pathogenicity in birds. It has been shown that VP2 mediates virus binding to DF-1 cells through heat shock protein (HSP) 90 (Lin et al., 2007). Studies have also demonstrated that VP2 may induce apoptosis *in vitro* (Fernandez-Arias et

al., 1997). However, the detailed molecular basis for the pathogenicity of very virulent IBDV (vvIBDV) is still poorly understood.

VP3 is an inner capsid protein that plays a major role in efficient encapsidation (Tacken et al., 2002). It is a group-specific antigen, carrying basic amino acids at its carboxy-terminal end. Anti-VP3 antibodies have been recognized by non-neutralizing antibodies (Martínez-Torrecuadrada et al., 2000) and can cross-react with serotype 1 and 2 strains (Becht et al., 1988b; Oppling et al., 1991). VP3 might also act as a scaffolding protein for VPX-VP2 processing, which is probably an essential step for the morphogenesis of IBDV particles (Tacken et al., 2002). This protein may participate in the induction of apoptosis at the early stage of infection. Busnadiego et al. (2012) demonstrated that VP3 inhibits apoptosis via the PKR-mediated pathway (Busnadiego et al., 2012). A recent study demonstrated that it plays a role in preventing the antiviral immune response, inhibiting the reaction of chicken melanoma differentiation-associated gene 5 (MDA5) to viral RNA in the host cells (Ye et al., 2014). Chen et al. (2016) showed that VP3 interacts with host ribosomal protein L4 (RPL4) (Chen et al., 2016).

VP4 is a minor, non-structural polypeptide. One of the most important functions of this protein is self-processing of poly-proteins (Azad et al., 1987; Birghan et al., 2000; Kibenge and Dhama, 1997). It contains a serine-lysine (Ser-652 and Lys-692) catalytic site which belongs to the Lon protease family. The cleavage site of the polyprotein is located at the C-terminus of VP2 (Lejal et al., 2000). The products of the IBDV poly-proteins after self-processing are VP2a, VP3, VP4 (Azad et al., 1987).

Table 1. Functions of IBDV proteins.

Function	Identified or predicted protein function	Reference
VP1	viral polymerase	(Sauger et al. 2010)
	virulence determinant	(Lenouen et al. 2012)
	encapsidation of viral particles	(Lombardo et al., 1999)
VP2	host receptor binding	(Ogawa et al. 1998)
	contains neutralizing epitopes	(Azad et al. 1987)
	virulence determinant	(Brandt et al. 2001)
	tissue-/ cell culture adaptation	(Mundt et al. 1999)
	induction of apoptosis	(Fernandezarias et al.1997)
	antigen variation	(Castón et al., 2001)
VP3	virion morphogenesis and encapsidation	(Lombardo et al. 1999)
	endopeptidase activity	(Irigoyen et al. 2009)
	chaperone activity	(Chevalier et al. 2004)
	antiapoptotic by interacting with PKR	(Busnadiego et al. 2012)
	the C-terminal region of VP3 in packaging is stabilizing the RNA genome within the interior of the capsid	(Chevalier et al. 2004)
VP4	suppresses hosts RNA silencing mechanisms	(Valli et al. 2012)
	transcriptional activator	(Tacken et al. 2002)
	forms ribonucleoprotein complex	(Luque et al. 2009)
	viral protein processing (viral protease), auto-processing of the polyprotein as a virus-encoded protease producing VP2,3,4	(Birghan et al. 2000)
	trans-active VP1 synthesis	(Birghan et al. 2000)
	suppresses type 1 IFN by interacting with GILZ	(Li et al. 2013)
	maturation of capsid protein VP2	(Lejal, 2000)
VP5	extensive accumulation within the plasma membrane	(Lombardo et al. 2000)
	early antiapoptotic effects	(Liu and Vakhria, 2006)
	late apoptotic effects	(Li et al. 2012)
	incriminated in the induced bursal pathology	(Mundt et al. 1997)
	role in virus dissemination	(Yao et al. 1998)
	regulatory function on virus release	(Mundt et al. 1997)

VP=Viral protein; IFN=interferon; PKR=Protein Kinase R; GILZ=glucocorticoid-induced leucine zipper protein. Content is modified from (Aregitu, 2015).

VP5 is a 17-k-Da nonstructural (NS) protein. It is a small protein encoded by the second ORF on segment A, which overlaps with the ORF encoding the N-terminal region of VP2 (Liu and Vakharia, 2006; Mundt et al., 1995). It is highly basic, cysteine-rich, and conserved among all serotype I IBDV strains (Yao et al., 1998). To date, several studies have focused on the role of VP5; however, the description of its properties is still controversial (Mundt et al., 1997; Yao et al., 1998). VP5 is not present in the virus particle, but accumulates in the host plasma membrane, inducing cell lysis or apoptosis and decreasing cellular viability (Mundt et al., 1997; Yao et al., 1998). Liu et al. (2006) indicated that VP5 inhibits apoptosis at early stages of viral infection (Liu and Vakharia, 2006). Later, it was demonstrated that VP5 activated PI3K/Akt signaling, resulting in the suppression of premature apoptosis (Wei et al., 2011). In another study, Lombardo et al. (2000) indicated that VP5 induces cell lysis (Lombardo et al., 2000). Li et al. (2012) suggested that VP5 is the major viral apoptosis inducer, playing a role in interactions with mitochondrial ionic channels (Li et al., 2012). VP5 knockout mutants could not induce clinical signs or induce bursal atrophy, suggesting that VP5 might act as a major IBDV virulence factor, playing a key role in viral pathogenesis (Qin et al., 2010). Wu et al. demonstrated that VP5 plays a role in viral release from infected cells, but it did not prevent intracellular virus production (Wu et al., 2009). Lombardo et al. (2000) demonstrated that VP5 might act as a type II transmembrane polypeptide with the N-terminal tail in the intracellular space and the C-terminal region exposed to the extracellular space (Lombardo et al., 2000). Recently it was suggested that VP5 may not be a type II transmembrane protein but an intracellular membrane-associated protein (Carballeda et al., 2015).

4.1.5. *IBDV antigenicity*

As determined via virus neutralization tests, two distinct serotypes of IBDV have been identified so far. They were designated serotype 1 and serotype 2. However, these two serotypes cannot be distinguished via immunofluorescence test or enzyme-linked immunosorbent assay (ELISA) (Eterradossi and Saif, 2008; Zierenberg et al., 2001). Only 30% antigenic similarity was observed among the serotype 1 strains (Ismail and Saif, 1991). Other studies indicated that only 33% antigenic relatedness was observed between two strains of serotype 2 (Eterradossi and Saif, 2008). While serotype 2 includes only non-pathogenic isolates, strains of serotype 1 may lead to disease. The degree of virulence varies between strains. There is no cross-protection between serotype 1 and serotype 2 strains (Eterradossi

and Saif, 2008). Serotype 1 is further categorized into: classical strains, antigenic variants, classical attenuated strains, and very virulent strains. The representative classical strains were first reported in Gumboro. They induce inflammation and lymphoid depletion in infected chickens, and lead to mortality rates of around 30%. Variant strains were first reported in the US in the 1980s (Mahgoub et al., 2012). These strains may induce a depletion of B cells in the BF and severe bursal atrophy. Attenuated strains are used as vaccine strains and do not cause any clinical disease, but may still induce bursal lesions with transient immunosuppression. vvIBDV was first reported in Europe in 1989 (Chettle et al., 1989b). Afterwards, these strains spread all around the world, except for New Zealand and Australia. Compared to the classical virulent IBDV strains (vIBDV), vvIBDV strains cause higher mortality rates, ranging from 60 to 100%, and more severe immunosuppression in susceptible birds, compared to mortality rates of 30 to 50% due to vIBDV strains (Mahgoub et al., 2012).

4.1.6. *Epidemiology*

The natural hosts of IBDV are chickens (Eterradossi and Saif, 2008). A serotype 1 virus was isolated from eight-week-old ostrich chicks (Mundt et al., 1995). The serotype 1 virus was also detected in healthy as well as dead waterfowl (McFerran et al., 1980). It was also isolated from captive penguins, pheasants, partridges, rooks, gulls, shearwater, quails and guinea fowl (Campbell, 2001; Gardner et al., 1997; Van den Berg et al., 2001). vvIBDV was isolated from a dead magpie (Jeon et al., 2008). It was demonstrated that crows and falcons are also susceptible to IBDV (Eterradossi and Saif, 2008). Japanese quails were shown to be refractory to IBDV infection (Greenfield et al., 1986; Tsukamoto et al., 1995). Dogs were suggested as potential carriers of the virus, since infective virus persisted in the feces for two days after initial ingestion (Spies and Müller, 1990).

IBDV is resistant to adverse environmental conditions, such as high pH, as well as a wide range of chemical treatments (Benton et al., 1967). It was demonstrated that the virus is less infective when treated with heat or ultraviolet light (Phillips and Opitz, 1995). It survives at 25°C for 21 days, at 56°C for as long as 5 hours, and at 60°C only for 90 minutes (Confer and MacWilliams, 1982). Viral infectivity is reduced when the virus is exposed to 0.5% formalin for 6 hours or 0.5% chloramine for 10 minutes (Benton et al., 1967). It is inactivated at pH=12 but not at pH=2 (Benton et al., 1967). After IBDV infection, chickens shed the virus

via feces for as long as 16 days (Winterfield et al., 1972). A chicken barn that housed IBDV-infected birds was still infective between 54 and 122 days after their removal (Benton et al., 1967). Feed, droppings, as well as water from IBDV-infected flocks contribute to viral dissemination (Benton et al., 1967). Dry fecal matter is shown to be the major component of airborne particles in broiler houses (Cambra-López et al., 2011). The sale of live chickens is related to quick dissemination of infection through bird-to-bird contact, haphazard disposal of visceral organs and feathers after slaughtering is also a culprit (Henzler et al., 2003). It was shown that contaminated equipment used by farmers and vendor vehicles on the farm premises are still strong risk factors for the spread of IBDV.

4.1.7. *Clinical disease*

Chickens are the only animal species that exhibits clinical disease and distinct lesions when exposed to IBDV. Experimental studies which focused on the pathogenesis of IBDV strains demonstrated that the breed, age, and immune status of exposed birds contribute to varying outcomes. Generally, the first clinical symptoms consist of ruffled feathers and white or watery diarrhea, followed by weakness, somnolence, drooping wings, anorexia, trembling, and severe prostration (Sharma et al., 1989). In some cases, the infected birds might also have a subnormal body temperature (Cosgrove, 1962; Islam et al., 2001).

The period of greatest susceptibility to clinical disease is between three and six weeks of age. Chickens less than three weeks old generally do not show IBD symptoms, but develop a severe immunosuppression that leads to an increased susceptibility to opportunistic pathogens as well as a poor response to widely used vaccines (Lucio and Hitchner, 1980; Wyeth, 1975). The reason for age dependence in the field might be interference from maternal derived antibodies (MDA). Birds infected with very virulent strains of IBDV show higher mortality rates compared to birds infected with classical IBDV strains (Chettle et al., 1989; Snyder et al., 1992). Mortality usually reaches a paek at the acute phase of disease at around three to five dpi and drops afterwards (Jung, 2006).

4.1.7.1. Pathology and histology

Classical macroscopic lesions are observed in primary lymphoid tissues such as the BF and thymus, in secondary lymphoid tissues like the spleen and CT, as well as in other tissues, namely intestine, liver, kidney, and muscles. Infected birds occasionally show dehydration of the breast and leg muscles and different degrees of hemorrhage in the legs, wings, and pectoral muscles (Cosgrove, 1962; Millar and Naqi, 1980; Naqi and Millar, 1979; Wang et al., 2009a).

The BF is the primary target organ for IBDV. Saif et al. (1998) indicated that the size of the bursa changes during the infection. As early as three dpi, it increases because of oedema and hyperaemia. By five dpi, it returns to a normal size, this is followed by atrophy due to the degeneration and necrosis of lymphocytes in the medullary area of the bursal follicles (Saif, 1998). The bursa may enter a gelatinous stage which causes it to appear yellowish by day two or three post-infection, and it may become cream gray during bursal atrophy (Hassan et al., 1996). Histological lesions in the bursa as degeneration and necrosis of lymphocytes in the medullary area of bursal follicles could be shown as early as one dpi (Eterradossi and Saif, 2008). The follicles are affected during infection and exhibit pyknotic debris as well as an infiltration of heterophils. Additionally, hyperplastic reticuloendothelial cells are observed at the early phase of infection, around three to four dpi (Naqi and Millar, 1979). During this stage of infection, scattered foci of repopulating lymphocytes are observed which do not develop into healthy follicles (Cheville, 1967; Helmoldt and Garner, 1964). It has been shown that IBDV induces a proliferation of the bursal epithelial layer which contains globules of mucin (Elankumaran et al., 2002; Sharma et al., 1989).

The thymus of IBDV-infected chickens exhibits a marked atrophy during the acute phase of infection (Tanimura and Sharma, 1997; Tsukamoto et al., 1992). It was shown that lymphocyte necrosis and hyperplasia of the reticular and epithelial components in the medullary regions of thymic follicles occur during the acute phase of IBDV infection (Helmboldt and Garner, 1964; Tanimura and Sharma, 1998). The thymus recovers to its normal status after a few dpi.

The spleen becomes slightly enlarged at the early stage of infection. During the infection, small gray foci may be uniformly dispersed on the surface of the spleen of IBDV-infected birds (Reddy et al., 1992). Although IBDV antigen might be found in the spleen

(Rautenschlein et al., 2007), virus-specific *ex vivo* proliferation of splenocytes was not detected (Kim et al., 2000). Apoptotic changes are shown in the spleen during IBDV infection (Jungmann et al., 2001). Birds recover from spleen lesions quickly without any sustained damage to the germinal centers (Elankumaran et al., 2002; Helmboldt and Garner, 1964).

CT may show effects after IBDV infection: acute heterophilic inflammation and lymphocyte depletion have been observed, and CT regenerates around five dpi (Helmboldt and Garner, 1964). IBDV antigen is mainly observed in the germinal centers of the CT during infection (Mertens et al., 1982).

It was demonstrated that IBDV induces a decrease in the number of plasma cells at seven dpi in the Harderian gland of one-day-old chickens as well as in adult birds (Helmboldt and Garner, 1964). The morphology of the Harderian gland recovers soon, and it was shown that the number of plasma cells reaches normal levels again at about 14 dpi (Dohms et al., 1981; Eterradossi and Saif, 2008; Survashe et al., 1979).

Kidneys of IBDV-infected birds do not show specific lesions. Only 5% of IBDV-infected chickens show an infiltration of heterophils, signs of dehydration, and nephrosis within swollen kidneys (Helmboldt and Garner, 1964; Icard et al., 2008).

The liver may be diffusely affected by IBDV, with congestion in the central veins and hepatic sinusoids as well as fatty degeneration of hepatocytes at three dpi. A slight perivascular infiltration of monocytes was also observed (Peters, 1967).

Lesions in the gut have not been widely studied. Occasionally, petechial hemorrhages might occur in the mucosa at the juncture of the proventriculus and gizzard (Cosgrove, 1962; Eterradossi and Saif, 2008; Wang et al., 2009a). According to Wang et al., IBDV could induce a decrease in the villus height in the ileum and jejunum as well as a decrease in the number of intestinal intraepithelial lymphocytes and mast cells (Wang et al., 2009b). An increase in the number of goblet cells contributes to more mucus production (Wang et al., 2009b).

4.1.8. *IBDV-pathogenesis*

The BF is the primary target organ of IBDV. IBDV replicates in macrophages and B cells in the bursa, and evidence of viral infection was observed within 24 hours post-infection (hpi) (Eterradossi and Saif, 2008). After oral infection or inhalation of IBDV, mononuclear phagocytic cells and lymphocytes from the intestinal mucosa get infected first. The virus initially replicates in lymphocytes and macrophages in the gut-associated lymphoid tissues (GALT) as early as four hpi (Fadly and Nazerian, 1983; Ley et al., 1983), and at five hpi, viral antigen can be detected in lymphocytes in the duodenum and jejunum (Ley et al., 1983). At the same time, the virus reaches the liver and enters the bloodstream, leading to a primary viremia. At 11 hpi, the virus starts replicating in proliferating B lymphocytes of the BF (Ley et al., 1983; Tanimura et al., 1995). At 13 hpi, most bursal follicles are virus-positive (Tanimura et al., 1995). The virus-infected cells then migrate into the portal circulation or distribute to different tissues via blood circulation, causing secondary viremia (Saif, 1998).

4.1.8.1. Host cells

The primary target site for extensive IBDV replication is the cytoplasm of intrabursal IgM+ B cells (Hiraga et al., 1994; Kaufer and Weiss, 1980). Virus particles can also be detected in the thymus, spleen, and CT, and it was shown that IBDV may disseminate to other lymphoid organs such as PP and Harderian glands (Eterradossi and Saif, 2008; Rautenschlein et al., 2007; Sharma et al., 2000; Williams and Davison, 2005). Mahgoub et al.,(2012) demonstrated that IBDV particles were detected in the CD8αα^{+} TCR2^{-}, CD4^{+} TCR2^{-}, CD4^{-} CD8αα^{-} TCR2^{+}, CD8αα^{+} TCR2^{+}, and CD4^{+} TCR2^{+} cells in the BF (Mahgoub et al., 2012). It was shown that bone marrow and CT may act as non-bursal lymphoid tissues supporting virus replication at later time points (Elankumaran et al., 2002).

4.1.8.2. Immunosuppression and immunomodulation

Allan et al. (1972) first reported that the immunosuppressive effect is one of most pronounced characteristics of IBDV, it occurs within the first two to three weeks post infection (Allan et al., 1972) and affects both humoral and cellular immunity (Faragher et al., 1974). Immunosuppressed chickens are more susceptible to secondary infections and show a lower feed conversion rate, weaker protective responses to vaccination, and higher rates of carcass condemnation at the processing level (Sharma et al., 2000).

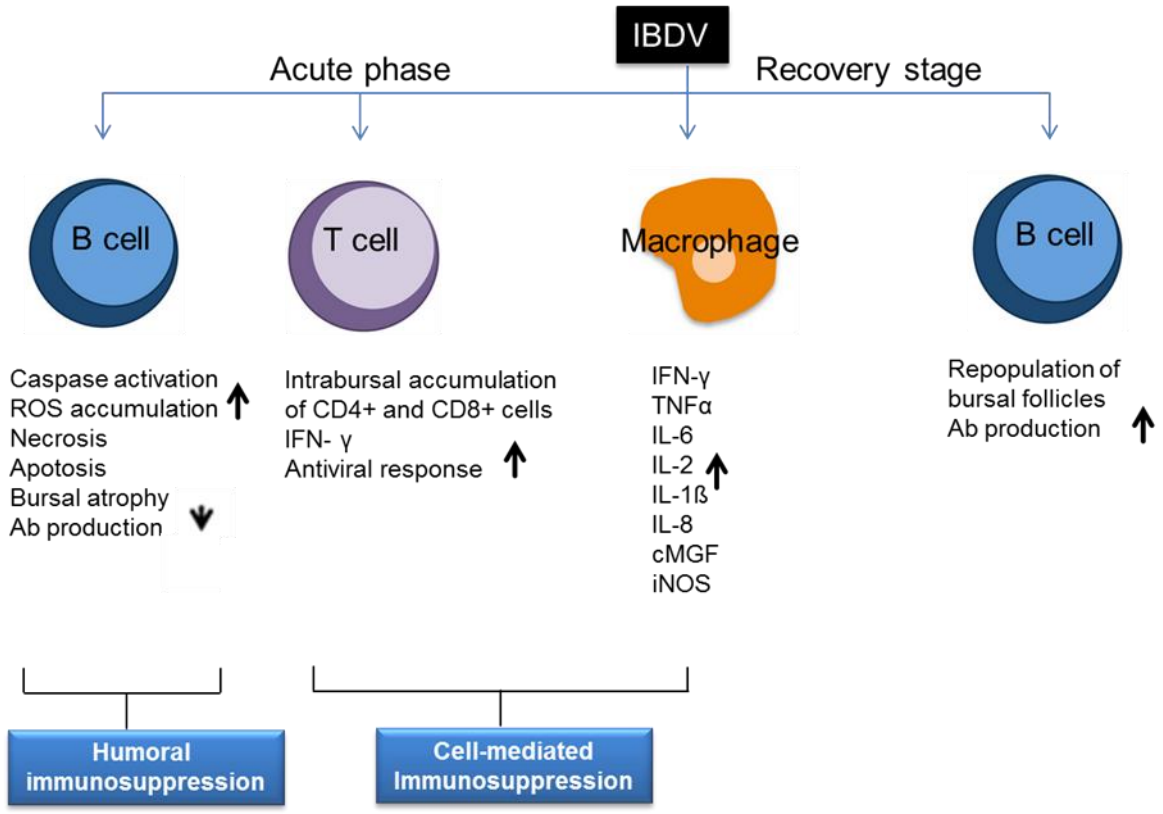

Figure 2: General aspects of IBDV-induced immunosuppression.

ROS=reactive oxygen species; Ab=antibody; IFN=interferon; cMGF=chicken myelomonocytic growth factor; IL=interleukin; iNOS=inducible nitric oxide synthase; ↑=upregulation; ↓=downregulation.

Figure is modified according to a review by Sharma el at. (2000).

The general aspects of IBDV-induced immunosuppression are summarized in Figure 2. The suppressive effect on the humoral immune system is associated with a reduction of circulating B cells in the peripheral blood of IBDV-infected chickens (Hirai et al., 1974). A depletion of lymphocytes in lymphoid organs, such as the spleen, thymus, and CT, is also observed in IBDV-infected chickens (Ivanyi and Morris, 1976; Rodenberg et al., 1994). IBDV targets immature sIgM B-lymphocytes, leading to a rapid depletion of B lymphocytes due to necrosis or apoptosis depending on the infecting strain (Rodríguez-Lecompte et al., 2005). IBDV modifies the IgM-producing B cells in a way that they fail to polymerize monomeric IgM (Ivanyi and Morris, 1976), and the virus leads to deficiencies of IgG (Hirai et al., 1974). Infected chickens produce lower levels of antigenic antibodies. Van den Berg et al. (2004) indicated that one-day-old chickens infected with IBDV exhibit a complete lack of serum

IgG, and only produce monomeric IgM (Van den Berg et al., 2004). Only primary antibody responses are affected by a prolonged suppression (Hirai et al., 1981; Sharma et al., 2000), while secondary responses remain intact (Hirai et al., 1981).

The effect of IBDV on cellular immunity is transient and not as pronounced as the effect on the humoral immunity. Previous studies showed that an infiltration of CD4+ and CD8+ T lymphocytes into the BF could be detected as early as one dpi, and peaked at around seven dpi in the BF during the run of the disease (Kim et al., 2000; Sharma et al., 2000). Rauf et al. (2010a) showed that cytotoxic T cells play a role in the clearance of IBDV-infected bursal cells (Rauf et al., 2012a). The activation of T cells may last up to 12 weeks post infection, while the IBDV antigen may have been cleared by 21 dpi (Mahgoub et al., 2012). IBDV particles were detected in intrabursal T cells, but no T cell depletion was observed in the bursa during IBDV infection (Mahgoub et al., 2012). Sivanandan and Maheswaran et al. (1980) indicated that the suppression of cellular immunity occurred six weeks post inoculation (Sivanandan and Maheswaran, 1980). T cells of infected chickens fail to respond properly to mitogens *in vitro* (Sharma et al. 1993). IBDV infections induced a poor cellular immune response to certain pathogens and increased the susceptibility to diseases that are under the control of the cellular immune defense (Anderdon et al., 1977; Confer et al., 1981).

Evidence of mRNA cytokines overexpression, such as of interleukin (IL) -6, IL-1ß, interferon (IFN)-γ and iNOS, indicated that IBDV could affect functions of macrophages. It was demonstrated that macrophages play a key role in innate immunity during IBDV infection. Khatri et al. (2006) showed that the activation of macrophages was through the NF-κB and p38 MAPK pathway during IBDV infection (Khatri and Sharma, 2006). An increase in the number of macrophages was observed in the BF of IBDV infected chickens, while a decrease in the number of macrophages was shown in the spleen (Palmquist et al., 2006). Palmquist et al. (2006) suggested that upregulation of the cytokines and a decrease in macrophage numbers in the BF might result in a decrease in the resident macrophages in the spleen (Palmquist et al., 2006). The upregulation in cytokines and macrophage activation induced by IBDV might delay the recovery process (Rauw et al., 2007).

Bursal recovery occurrs in the IBDV-infected chickens. It was shown that the repopulation occurred faster in the chickens exposed to an IBDV-vaccine strain (IBDV-Vac) than in the chickens exposed to a virulent IBDV strain (IM-IBDV) (Kim et al., 1999). By about seven

weeks pi, 40% and 80% of bursal follicles in IM-IBDV-and IBDV-Vac-inoculated chickens, respectively, were repopulated with immunoglobulin (Ig) M+ B lymphocytes (Kim et al., 1999). There are two different types of recovered follicles: large and small follicles (Withers et al., 2005). Large follicles have normal structures with rapidly proliferating B cells. The small follicles lack a distinct cortex and medulla, and are unable to produce antigen-responsive B cells and are incapable of gene conversion or emigration of B cells to the periphery. It was suggested that the overall percentage of small follicles determined the degree of immunosuppression (Withers et al., 2006; Withers et al., 2005).

4.1.9. *Immune response to IBDV*

IBDV infection leads to an activation of all branches of the immune system. During the acute phase, an infiltration of immune cells induces a strong inflammatory response. Subsequently, due to a lack of regulation, a ‘‘cytokine storm’’ may be caused. These immune responses may contribute to tissue destruction, impede recovery, and therefore harm the host (Khatri et al., 2005; Rautenschlein et al., 2007). It was also suggested that the cytokine storm may be the reason for the high death rate in infected birds (Berg, 2000b; Jung, 2006).

4.1.9.1. Innate immunity

During the acute phase of IBDV infection, the viral replication leads to a pronounced decrease in the number of B cells due to necrosis and apoptosis. An infiltration of macrophages, heterophils, and mast cells can be observed in the BF, this is an indication of an early innate immune response to IBDV (Khatri et al., 2005; Palmquist et al., 2006; Rautenschlein et al., 2007; Wang et al., 2008b). IBDV did not affect the cytotoxicity and mitogenic response of natural killer (NK) cells (Sharma and Lee, 1983). A transient early depression of NK cells was observed in IBD infected chickens (Kumar et al., 1998). Later, a downregulation of gene expression of NK lysin was observed in Rauf et al.’s study, in which they suggested that NK cells might not be involved in mediating the cytotoxic response against IBDV (Rauf et al., 2011c). In one recent study, microarray analysis indicated an upregulation of NK cell-lysin in the bursa at three and four dpi, which suggested that NK cells are involved in the response to the virus (Smith et al., 2015)

Host cells recognize viruses via pattern recognition receptors through pathogen-associated molecular patterns (PAMPS), and subsequently mount an antiviral response. Toll-like

receptors (TLRs) are an important group of pattern recognition receptors (Kawai and Akira, 2006). So far, IBDV has been shown to induce an immune response through the activation of TLR3 *in vivo* (Rauf et al., 2011a). Furthermore, studies demonstrated an upregulation of TLR3, TLR4, and TRIF in IBDV-infected chickens (Guo et al., 2012; Lee et al., 2015; Rauf et al., 2011a) and in chicken embryo fibroblast (CEF) cells (Wong et al., 2007). It was shown that IBDV infection induces a downregulation of TLR2B, TLR7, and MyD88 in the BF (Guo et al., 2012; Rauf et al., 2011a). The downregulation of TLR2B may contribute to the suppression of the immune response (Guo et al., 2012). Additionally, Ye et al. demonstrated that VP3 of IBDV has a high affinity to the chicken MDA5 and thus blocks the induction of the signaling pathway to IBDV genomic dsRNA, which results in a failure to recognize the viral RNA and prevents the antiviral immune response (Ye et al., 2014).

During infection, the local inflammatory response recruits phagocytic and non-phagocytic lymphoid cells. Interactions between IBDV and host cells result in different gene expression patterns, which vary depending on virus strains, age of birds, and infected cell type (Kim et al., 1998; Lee et al., 2015; Rasoli et al., 2015; Wang et al., 2008b; Yasmin et al., 2016). Generally, gene expressions involved in the innate immune response such as MD-1 and MD-2, complement components, heat shock protein (HSP) 70, and HSP47 have been investigated in *in vivo* and *in vitro* (Eldaghayes et al., 2006; Mo et al., 2001). Additionally, IBDV infection upregulates MHC class I and II mRNA expression in HD11 and chicken embryo cell (CE) cultures (Rasoli et al., 2015). Different cytokines, including members of the antiviral interferon system (Ye et al., 2014) and proinflammatory cytokines (IL-1ß, IL-2, IL-6, IL-18, IL-12), as well as chemokines (as IL-8, MIP-1a/1ß), are induced by IBDV during the early infection (Lee et al., 2015). Tables 2-5 provide a summary of IBDV induced cytokines as well as chemokines *in vivo* and *in vitro*. Pro-inflammatory cytokines are a natural response to infection and may be beneficial to host defense. A temporary upregulation of IFN-γ and type I IFNs was noted in IBDV infection (Eldaghayes et al., 2006; Rautenschlein et al., 2007). Eldaghayes et al. (2006) demonstrated that IBDV infection inhibited the production of IFN-I in chickens (Eldaghayes et al., 2006) and Ye et al. (2014) showed that it did not induce the production of IFN-I, either *in vivo* nor *in vitro* (Ye et al., 2014). During IBDV infection, an upregulation of IL-8 was observed which activatied through the MAPK and NF-kB pathways (Khatri and Sharma, 2006; Kim et al., 1998). The effect of IL-8 is thought to be to attract and

activate macrophages and leukocytes, which may contribute to the inflammatory responses in the BF (Fleckenstein, 2001).

4.1.9.2. Acquired immune response

Humoral immunity

Humoral immunity plays a crucial role in protection against IBDV. Natural or experimental IBDV infection induces high titers of circulating IBDV-specific antibodies in chickens (Eterradossi and Saif, 2008). A few weeks after IBDV infection, all classes of antibodies are increased in the sera (Aricibasi et al., 2010; Eterradossi and Saif, 2008; Maas et al., 2001).

The depletion of B cells in the BF and peripheral blood leads to an adverse effect on the antibody response to other pathogens or vaccines, shown through IBDV-induced IgG suppression that may vary based on the age of the bird at IBDV challenge (Sharma et al., 2000). Chickens infected with the IBDV at day one post hatch were completely deficient in serum IGG and produced only a monomeric IgM (Ismail et al., 1990; Van den Berg et al., 2004).

MDA provides protection in the first few weeks after hatching (Alnatour et al. 2004). Studies demonstrated that MDA-positive chickens developed bursal lesions after an IBDV challenge, but they were less severe compared to MDA-negative chickens (Hassan et al. 2002; Aricibasi et al. 2010). Although antibody mediated immunity is crucial for protection against IBDV, an important role of the cell-mediated immunity (CMI) was suggested (Rautenschlein et al. 2002; Yeh et al. 2002). Chickens with severely compromised antibody producing ability following treatment with cyclophosphamide showed sufficient protection against IBDV challenge despite the absence of detectable vaccine-induced antibodies (Yeh et al. 2002). Rautenschlein et al. (2005) suggested that MDA may affect the development of an active immune response after IBDV vaccination (Rautenschlein et al. 2005).

Cellular immunity

The role of CMI in the control of IBD has been studied in vaccination studies with T cell-or B-cell-compromised chickens. Rautenschlein et al. (2002b) observed that T cell-compromised chickens after neonatal thymectomy or Cyclosporin A treatment developed lower protection rates after immunization with an inactivated IBDV vaccine in comparison to T cell-intact chickens (Rautenschlein et al. 2002b). Activated T cells may be detected up to 12 weeks post-

infection, while the IBDV antigen may have been cleared already by 21 dpi (Mahgoub et al., 2012). Rauf et al. (2011b) indicated that the infiltration of T cells in the bursa correlated with higher levels of perforin (PFN) and granzyme A (Gzm A) mRNA expression, which are known to play a role in cytotoxic activity and virus clearance (Rauf et al. 2011b). Additionally, it was confirmed that cytotoxic T cells play a role in the clearance of IBDV-infected bursal cells (Rauf et al., 2012a).

Table 2: *In vivo* investigations of associated molecules of the innate and acquired immune responses during IBDV infection in chickens

Cytokine/ Receptor/ Substance	Regulation of expression levels	Cell/Organ/ Sample	Virus	Reference
CXCLi1	↑at 2, 4 and 5 dpi	spleen	vvIBDV	(Rasoli et al., 2015)
	↑at 4 and 5 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
CCL4	↑at 2, 4 and 5 dpi	spleen	vvIBDV	(Rasoli et al., 2015)
	↑at 4 and 5 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
chCCLi21	↑at 4 dpi	bursa	vIBDV	(Khatri et al., 2005)
chCCLi6	↑at 2 dpi	bursa	vIBDV	(Khatri et al., 2005)
TLR 3	↓at 4 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
	↓at 3, 5 and 7 dpi	bursa	vIBDV	(Rauf et al., 2011)
	↑at 3, 5 dpi and ↓at 7 dpi	bursa	cIBDV	(Rauf et al., 2011)
TLR 7	↓at 2 and 4 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
	↓at 3 and 5 dpi	bursa	vIBDV	(Rauf et al., 2011)
IL-8	↑at 3, 5 and 7 dpi	bursa	vIBDV, cIBDV	(Rauf et al., 2011)
	↑at ED 21	thymus, spleen	cIBDV, aIBDV	(Maccallum et al., 2006) (Khatri et al., 2009)
	↑at 2, 4 and 5 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
MIP-α	↓at 3 dpi and ↑at 5 and 7 dpi	bursa	vIBDV	(Rauf et al., 2011)
	↑ at 3 and 7 dpi	bursa	cIBDV	(Rauf et al., 2011)
TGF-β3	↓at 2 dpi	spleen	vvIBDV	(Rasoli et al., 2015)
	↓at 4 and 5 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
TGF-β4	↓48–96 hpi	bursa	vIBDV,vvIBDV	(Shaughnessy et al., 2009)
MHCI	↑at 4 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
MHCII	↑at 4 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
Cox-2	↑within 4 and 8 hpi	macrophage	IM-IBDV	(Khatri et al., 2006)

table 2: continuing

iNOS	↑at 2, 4 and 5dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
	↑at 3 and 5 dpi	spleen	vvIBDV	(Tippenhauer et al., 2013)
	↑at 2, 3 and 5dpi	bursa	vvIBDV	(Tippenhauer et al., 2013)
	↑at 5 dpi	spleen	vvIBDV	(Khatri et al., 2005)
	↑at 1 dpi	splenocyte	IM-IBDV	(Palmquist et al., 2006)
Beta-defensin	↓at 3 dpi	bursa	vvIBDV	(Raj et al., 2011)
TNFSF13B	↑at 4 and 5 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)

vvIBDV=very virulent IBDV; cIBDV=classical IBDV; IM-IBDV=virulent IBDV; TLR=Toll-like receptor; CXCLi=Chemokine (C-X-C Motif) Ligand; TNFSF=tumor necrosis factor super family; MHC=major histocompatibility complex class; MIP=macrophage inflammatory proteins; COX=cyclooxygenase; IL=interleukin; TGF=transforming growth factor; iNOS=inducible nitric oxide synthase; h(d)pi=hours (days) post-IBDV inoculation; ↓=downregulation; ↑=upregulation.

Table 3: *In vivo* investigations of cytokine induction after IBDV infection in chickens

Cytokine	Regulation of expression levels	Organ/Sample	Virus	Reference
IL-1 β	↑at 2, 4 and 5 dpi	spleen	vvIBDV	(Rasoli et al., 2015)
	no change	bursa	vvIBDV	(Rasoli et al., 2015)
	↑at 36, 48, 72 and 96 hpi	bursa	vIBDV	(Eldaghayes et al., 2006)
	↓ at 24 hpi, ↑ at 96 hpi	bursa	vvIBDV	(Eldaghayes et al., 2006)
	↑at 5 dpi	circulation	vIBDV, vvIBDV	(Eldaghayes et al., 2006)
IL-2	↓at 4 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
	↑at 4 dpi	bursal T cells	vIBDV, vvIBDV	(Eldaghayes et al., 2006)
	undeteced	bursa	vIBDV, vvIBDV	(Eldaghayes et al., 2006)
	↑at 2 dpi	bursa	IM-IBDV	(Rautenschlein et al., 2003)
IL -4	↑at 1 and 3 dpi	bursa	vvIBDV	(Liu et al., 2010)
IL-5	↑at 3 and 5 dpi	bursa	vvIBDV	(Liu et al., 2010)
	↓at 3 dpi and ↑at 5 dpi	bursa	ts IBDV	(Liu et al., 2010)
IL-6	↑at 2, 4 and 5 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
	↑at 3 and 5 dpi	serum	vvIBDV	(Rue et al., 2011)
	↑at 3, 5 and 7 dpi	bursa	cIBDV	(Rauf et al., 2011)
	↑atED 21	thymus, spleen	cIBDV, aIBDV	(Khatri et al., 2009)
	↑at 2 dpi	circulation	vIBDV	(Eldaghayes et al., 2006)
	↑at 60, 72 and 96 dpi	bursa	vIBDV	(Eldaghayes et al., 2006)
	↓at 24 hpi, ↑at 60, 96 dpi	bursa	vvIBDV	(Eldaghayes et al., 2006)
IL-10	↑at 5 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
	↑at 3 and 5 dpi	bursa	vvIBDV, tsIBDV	(Liu et al., 2010)
		bursa	tsIBDV	(Liu et al., 2010)
IL-12α	↑at 2, 4 and 5 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
	↑from 48 to 96 hpi	bursa	vIBDV, vvIBDV	(Eldaghayes et al., 2006)
IL-12ß	undetectable	bursa	vIBDV, vvIBDV	(Daghayes et al., 2006)
IL-13	↑at 3 and 5 dpi	bursa	vvIBDV	(Liu et al., 2010)
	↓at 3 dpi and ↑ at 5 dpi	bursa	tsIBDV	(Liu et al., 2010)
IL-18	↑at 2, 4 and 5 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
	↑at 5 dpi	splenocyte	IM-IBDV	(Palmquist et al., 2006)

IL-15	↑at 5 dpi	spleen	vvIBDV	(Rasoli et al., 2015)

table 3: continuing

IL-16	↓at 3 dpi	bursa	vvIBDV	(Raj et al., 2011)
	↓at 4 dpi	bursa	vvIBDV	(Rasoli et al., 2015)
	↓at 4 dpi	spleen	vvIBDV	(Rauw et al., 2007)
IL-17 F	↑at 5 dpi	spleen	vvIBDV	(Rasoli et al., 2015)
IL-18	no change	bursa	vIBDV, vvIBDV	(Eldaghayes et al., 2006)
IFN-γ	↑at 2, 4 and 5 dpi	spleen, bursa	vvIBDV	(Rasoli et al., 2015)
	↑till 4 dpi	bursa	vvIBDV	(Aricibasi et al., 2010)
	↓at 2 dpi	serum	vvIBDV	(Tippenhauer et al., 2013)
	↑at first 5 dpi	serum	vvIBDV	(Rue et al., 2011)
	↑at 2, 3 and 5 dpi	bursa, spleen	vvIBDV	(Tippenhauer et al., 2013)
	↑at 48 hpi	bursa	vvIBDV	(Daghayes et al., 2006)
	↑at 36 hpi	bursa	vIBDV	(Daghayes et al., 2006)
	↑at 5 hpi	spleen,bursa	vvIBDV	(Rauw et al., 2007)
	↑from 48 to 96 hpi	bursa	vIBDV	(Eldaghayes et al., 2006)
	↑from 36 to 96 hpi	bursa	vvIBDV	(Eldaghayes et al., 2006)
	↑at 2-5 dpi	serum	vvIBDV	(Rauw et al., 2007) (Rue et al., 2011)
	↑at 2 dpi	bursa	IM-IBDV	(Rautenschlein et al., 2003)
	↑at 3 and 5 dpi	bursa	IBDV-B2	(Rautenschlein et al., 2003)
	↑at 3 dpi	bursa	IM-IBDV	(Rautenschlein et al., 2003)
	↑at 21 ED	thymus, spleen	cIBDV, aIBDV	(Khatri et al., 2009)
IFN-α	↑at 3, 5 and 7 dpi	bursa	vIBDV	(Rauf et al., 2011)
	↓at 3 and ↑ at 5 and 7 dpi	bursa	cIBDV	(Rauf et al., 2011)
	↓at 60, 84 and 96 hpi	bursa	vIBDV	(Eldaghayes et al., 2006)
	↓at 24, 36, 84 and 96 hpi	bursa	vvIBDV	(Eldaghayes et al., 2006)
IFN-ß	↑at 3, 5 and 7 dpi	bursa	cIBDV, vIBDV	(Rauf et al., 2011)
	↓at 24 and 36 hpi and ↑at 60 and 96 hpi	bursa	vvIBDV	(Eldaghayes et al., 2006)
	no change	bursa	vIBDV	(Eldaghayes et al., 2006)

vvIBDV=very virulent IBDV; cIBDV=classical IBDV; tsIBDV=cell-adapted IBDV; IM-IBDV=virulent IBDV; IBDV-B2=IBDV Bursine 2 intermediate vaccine virus; IBDV-D78=IBDV vaccine strain D78; aIBDV=an attenuated strain of IBD; IFN=interferon;

IL=interleukin; ED=Embryonation day; ↑=upregulation; ↓=downregulation; h(d)pi=hours (days) post-IBDV inoculation.

Table 4: *In vitro* investigations of associated molecules of the innate and acquired immune responses during IBDV infection

Cytokine/ Receptor/ Substance	Regulation of expression levels	Cell	Virus	Reference
CXCLi1	↑at 6 and 24 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
CXCLi2	↑from 6 to 48 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
	↑at 3 and 6 hpi	BMDCs	vvIBDV	(Yasmin et al., 2015)
	↑at 1 and 3 hpi	PBMCs	IM-IBDV	(Jain et al., 2013)
ChCCLi4	↑at 1 and 24 phi	PBMCs	IM-IBDV	(Jain et al., 2013)
TLR 3	↑at 24 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
	↑at 6, 12 and 24 hpi	BMDCs	vvIBDV	(Yasmin et al., 2015)
	↑at 48 hpi	CEF	IBDV-D78	(Wong et al., 2007)
TGF-β3	No change	HD11	vvIBDV	(Rasoli et al., 2015)
TGF-β2	↑at1, 2 and 3 hpi	PBMCs	IM-IBDV	(Jain et al., 2013)
MHCI	↓at 24 hpi but ↑at 48 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
	↓at 2 at 8 hpi and ↑at 24 hpi	HD11	ts IBDV	(Lee et al., 2015)
	↑from 1 to 7 dpi	CE	IBDV-B2	(Li et al., 2007)
MHCII	↓at 8 hpi	HD11	ts IBDV	(Lee et al., 2015)
	↓at 2 hpi and ↑at 8, 16 and 24 hpi	HD11	ts IBDV	(Lee et al., 2015)
	↑at 12 and 24 hpi	BMDCs	vvIBDV	(Yasmin et al., 2015)
	↓at 24 and 48 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
	↑from 1 to 7 dpi	CE	IBDV-B2	(Li et al., 2007)
IL-8	↑at 24 hpi	HD11	ts IBDV	(Lee et al., 2015)
	↑at 4, 8 and 24 hpi	SM	vvIBDV	(Khatri et al., 2006)
	↑from 1 to 7 dpi	CE	IBDV-B2	(Li et al., 2007)
	↑at 1 dpi	BM	cIBDV, aIBDV	(Zheng et al., 2008)
	↑at 1 dpi	BM	vvIBDV	(Watson et al., 2005)
	↑at 3 dpi	SM	IM-IBDV	(Kim et al., 1998)
iNOS	↑from 6 to 48 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
	↑at 4, 8 and 24 hpi	SM	vvIBDV	(Khatri et al., 2006)

table 4: continuing

COX-2	↑at 4, 8 and 24 hpi	SM	vvIBDV	(Khatri et al., 2006)
MIP-3a	↑from 2 to 7 dpi	CE	IBDV-B2	(Li et al., 2007)

CE=chicken embryo cell; CEF=chicken embryo fibroblasts; SM=spleen macrophage; PBMCs=peripheral blood mononuclear cells; BMDCs=bone marrow derived dendritic cells; vvIBDV=very virulent IBDV; cIBDV=classical IBDV; tsIBDV=cell-adapted IBDV; IM-IBDV=virulent IBDV; MIP=macrophage inflammatory protein; TLR=toll-like receptor; CXCLi=chemokine (C-X-C Motif) Ligand; MHC=major histocompatibility complex class; COX=cyclooxygenase; NO=nitric oxide; TGF=transforming growth factor; iNOS=inducible nitric oxide synthase; ↑=upregulation; ↓=downregulation; h(d)pi=hours(days) post-IBDV inoculation.

Table 5: *In vitro* investigations of the cytokine induction after IBDV infection in cell cultures

Cytokine	Regulation of expression levels	Cell type/line	Virus	Reference
IL-1β	↑at 6, 24 and 48 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
	↑at 8 and 16 hpi	HD11	ts IBDV	(Lee et al., 2015)
	↑at 6 to 24 hpi	BM-DCs	vvIBDV	(Yasmin et al., 2015)
	↑at 1 and 3 dpi	BM	IM-IBDV	(Khatri et al., 2005)
	↑at 3 and 5 dpi	BM	IM-IBDV	(Khatri et al., 2007)
	↑at 3 dpi	BM	vvIBDV	(Rasoli et al., 2015)
	↑at 5 dpi and 7 dpi	SM	IM-IBDV	(Palmquist et al., 2006)
IL-2	↑at1, 2, 3, 24 and 48 hpi	PBMCs	IM-IBDV	(Jain et al., 2013)
GMCSF	↓at 12 hpi	PBMCs	IM-IBDV	(Jain et al., 2013)
IL-6	↑at 8, 16 and 24 hpi	HD11	tsIBDV	(Lee et al., 2015)
	↑from 1, 2 and 3 dpi	CE	IBDV-B2	(Li et al., 2007)
	↑at 1, 3, 6 and 24 hpi	PBMCs	IM-IBDV	(Jain et al., 2013)
	↑at 3 dpi	BM	IM-IBDV	(Khatri et al., 2005)
	↑at 1, 3 and 5 dpi	SM	IM-IBDV	(Palmquist et al., 2006)
	↑at 3 and 5 dpi	BM	IM-IBDV	(Khatri et al., 2007; Kue et al., 2011)
	↑at 1 dpi	SM	IM-IBDV	(Kim et al., 1998)
IL-10	↓at 6 and 24 hpi	HD	vvIBDV	(Rasoli et al., 2015)
	↑at 8, 16 and 24 hpi	HD11	tsIBDV	(Lee et al., 2015)
	↑from 0.5 to 48 hpi	PBMCs	IM-IBDV	(Jain et al., 2013)
IL-12	IL-12α ↑at 6, 12 and 24 hpi	BM-DCs	vvIBDV	(Yasmin et al., 2015)
	↑at 2 and 16 hpi	HD11	tsIBDV	(Lee et al., 2015)
	IL-12α↑at 6 and24 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
IL-16	↓at 24 hpi	HD11	vvIBDV	(Rasoli et al., 2015)

IL-18	↑at 3, 6, 12 and 24 hpi	BM-DCs	vvIBDV	(Yasmin et al., 2015)
	↑at 6 and 24 hpi	HD11	vvIBDV	(Rasoli et al., 2015)
	↑at 3 dpi and ↓ at 5 dpi	BM	IM-IBDV	(Khatri et al., 2007)
	↑at 24 hpi	HD11	tsIBDV	(Lee et al., 2015)
IFN-α	No change	CE	IBDV-B2	(Li et al., 2007)

table 5: continuing

IFN-ß	No change	CE	IBDV-B2	(Li et al., 2007)
IFN-γ	↑at 6, 12 and 24 hpi	BM-DCs	vvIBDV	(Yasmin et al., 2015)
	↑at 1 and 3 dpi	CE	IBDV-B2	(Li et al., 2007)
	↑at 0.5 and 48 hpi	PBMCs	IM-IBDV	(Jain et al., 2013)

CE=chicken embryo cells; CEF=chicken embryo fibroblasts; SM=spleen macrophage; PBMCs=peripheral blood mononuclear cells; BMDCs=bone marrow derived dendritic cells; vvIBDV=very virulent IBDV; IBDV-B2=IBDV Bursine 2 intermediate vaccine virus; cIBDV=classical IBDV; tsIBDV=cell-adapted IBDV; IM-IBDV=virulent IBDV; IFN=interferon; IL=interleukin; ED=embryonation day; ↑=upregulation; ↓=downregulation; h(d)pi=hours (days) post-IBDV inoculation.

4.1.10.*IBDV and co-infecting pathogens*

Immunosuppression relates to the state of transient or permanent dysfunction of the immune system and increases the susceptibility to other pathogens (Saif, 1991). The immunosuppressive effects of IBDV in infected birds have been widely studied using various secondary pathogens. Table 6 provides a summary of the interactions of IBDV with other pathogens. Salmonella, *Escherichia coli* (Wyeth, 1975), *Staphylococcus aureus* (Santivatr et al., 1981), coccidia (Anderson et al., 1977; Onaga et al., 1989), *C. jejuni* (Stojanov et al., 2008; Subler et al., 2006), Marek's disease virus (MDV) (Cho, 1970b; Sharma, 1984), infectious bronchitis virus (IBV) (Pejkovski et al., 1979), infectious laryngotracheitis virus (ILV) (Rosenberger and Gelb Jr, 1978), Newcastle disease virus (NDV) (Sultan et al., 2016), reovirus (Moradian et al., 1990), and *Mycoplasma synoviae* (Faragher et al., 1974) have been studied in recent studies. Most of the studies demonstrated that IBDV compromised the subsequent vaccine response. It was shown that IBDV-infected chickens were more

susceptible to other pathogens, such as NDV or chicken infectious anaemia virus agent (CIAV) (Faragher et al., 1974). The adverse effect of other pathogens on the IBDV-pathogenesis was not studied much. In one study, Cloud et al. (1992b) showed that infection with CIAV in IBDV-infected birds caused an adverse effect on the birds' immune system, such as thymus, spleen, and bone marrow, and the severity of clinical signs was increased (Cloud et al., 1992b).

Table 6: Coinfection of chickens with IBDV and other pathogens

Pathogen	The effect of IBDV on	Reference
Escherichia coli	mortality↑, severity of septicaemic lesions↑, lymphocytic depletion↑	(Nakamura et al., 1990)
	susceptibility to *Escherichia coli* infection↑	(Wyeth,1975)
Salmonella sp.	shedding↑, antibody level↓, delay of *Salmonella* clearance↓	(Bautista et al., 2004)
	lesion↑, anti-*Salmonella* IgG seroconversion↓, humoral immunity for *Salmonella* clearance↓.	(Bautista et al., 2004)
	susceptibility to salmonellosis infection↑	(Wyeth,1975)
Staphylococcus aureus	development of anti-nuclease antibody in younger birds↑, but insufficient protection	(Rodgers et al., 2006)
	susceptibility to *Staphylococcus* aureus↑, humoral defects↑	(Santivatr et al., 1981)
C. jejuni	colonization↑, shedding↑	(Subler et al., 2006)
	isolation from the liver↑, gut lesions↑, neutralization antibody level↓	(Stojanov et al., 2008)
Infectious bronchitis virus (IBV)	susceptibility to IBV infection↑, virus neutralizing antibodies in the serum↓	(Winterfield et al., 1978)
	mortality↑, lesions↑ duration of virus infection↑	(Gallardo et al., 2012)
	resistance to virus↓, antibody level↓	(Giambrone,1979)
Avian leukosis virus	no effect on the infection or antibody production	(Williams et al., 2012)
Infectious larygotracheitis virus (ILV)	prolong the persistant ILV infection↑	(Rosenberger et al., 1978)
Marek's disease virus (MDV)	susceptibility to MD↑, MD lesions↑	(Giambrone et al., 1976)
	susceptibility to MD↑	
	Incidence and severity of gross and nerval lesions↑	(Cho, 1970a)
	antiviral immune response↓	(Jen et al., 1980)

	no effect on the MD vaccination	(Jen et al., 1980)
Newcastle disease virus (NDV)	antibody response to ND vaccination↓	(Giambrone, 1979)
	immune response to ND vaccine↓	(Roussan et al., 2007)
	no effect on ND vaccination	(Otim et al., 2005)
Influenza viru (IV)	AIV antibody levels↓(H9N2)	(Motamed et al., 2013)
	susceptibility to AIV infections↑ (H5N2)	(Ramirez-Nieto et al., 2010)

table 6 :continuing

Reovirus	virus-neutralizing antibody↓, inflammation↑	(Springer et al., 1983)
Chicken infectious Anemia virus (CIAV)	interference with the transcription of ChIFN mRNA	(Ragland et al., 2002)
	primary CIAV infection↑	(Imai et al., 1999)
	persistance of CIAV↑	
	persistent depression of Ia-expressing cells in the bursa and the spleen	(Cloud et al., 1992a)
	anemia↑, chicken death rate↑	(Yuasa et al., 1980)
	delayed repopulation of the thymus	(Toro et al., 2009)
Eimeria tenella	histological lesions↑	(Faragher et al., 1974)
	mortality↑, lesion scores↑, hemorrhages↑	(Giambrone et al., 1977)
	protection against coccidial challenge↓	(Anderson et al., 1977)

MD=Marek's disease; CAV=Chicken Anemia virus; AIV=Avian influenza virus;

IBV=Infectious bronchitis virus; ND=Newcastle disease; ChIFN=chicken interferon.

4.1.11.*Prophylatic strategies: Vaccines for protection against IBDV*

Effective vaccination plays an important role in the successful control of this disease, along with the application of biosecurity measurements (Müller et al., 2012). To date, inactivated/killed, live attenuated recombinant, and immune complex vaccines are the most commonly used vaccines.

Inactivated/killed vaccines

Inactivated/killed vaccines are used for breeder vaccination to boost the level of MDA and prolong the duration of humoral immunity. Inactivated IBD vaccines may contain classical standard and/or variant strains, in order to induce immunity in breeders and in turn protect their progeny from infection by both virus types (Müller et al., 1992; Rosenberger et al., 1987). Usually, inactivated/killed vaccines are used as prime-boost vaccines, while attenuated live IBDV vaccines are used first for priming (Müller et al., 2012).

Conventional live attenuated IBDV vaccines

Conventional live attenuated IBDV vaccines consist of mild, intermediate, intermediate plus, or hot IBDV strains. These strains are developed by serial passages using eggs, embryo-derived tissues, or tissue cultures. They are administered mainly via the animals' drinking water. The virulence of these vaccines depends on the breakthrough titer and the level of MDA (Jung, 2006). ''Mild'' vaccines cannot break through high titers of MDA. Vaccinated chickens do not show any lesions, but these vaccines do not provide full protection against the disease. ''Intermediate'', ''intermediate plus'', and ''hot'' vaccines may have different breakthrough titers for MDA, but they may induce bursal lesions to some extent and, thus, induce transient immunosuppression (Kumar and Charan, 2001; Mazariegos et al., 1990; Rautenschlein et al., 2005; Tsukamoto et al., 1995). Experimental studies showed that these vaccines may not provide complete protection against vvIBDV (Rautenschlein et al., 2005) or antigenic variant strains. The advantage of live vaccines is that they replicate and induce both

cellular and humoral immunity. However, they may also have undesirable side effects, such as reversion to virulence due to mutations or residual immunosuppressive effects causing clinical disease, as well as their role as a genetic source for the generation of reassortant new viruses (Schijns et al., 2008).

The major problem with active immunization of young chickens is estimating the proper time of vaccination. It varies with the level of MDA and the route of vaccination (de Wit, 2001). Environment stresses and management as well as field pressure also should be taken into consideration (Hafez et al., 2003; Jung, 2006). Inactivated vaccines are used in birds which have been already stimulated by primary exposure, either to live vaccines or to field virus. Generally, live vaccines are applied during the first three weeks post hatch. Their application is based on the virulence and antigenic diversity of the respective vaccine strains of IBDV that are involved, as well as the high field pressure. It has been shown that the best IBDV vaccination schedule can be determined by calculating the optimal vaccination time with the Deventer formula (de Wit, 2001).

IBD immune complex (ICX) vaccines

ICX vaccines consist of a mixture of certain amounts of IBDV-specific antibodies obtained from IBDV-hyper immune sera of immunized chickens and of replicating IBDV (Johnston et al., 1997; Whitfill et al., 1995). Experimental studies have shown that ICX vaccines are safe and efficiently induce protection after *in ovo* and post-hatch vaccination (Iván et al., 2005). These ICX vaccines release the virus when the MDA titers decline, and induce a specific humoral immune response that protects the vaccinated chickens against a challenge.

Next generation vaccines

Most of the new-generation vaccine candidates are still under experimental investigation. VP2 as the immunodominant viral capsid protein, the VP4-2-3 polyprotein, the polyprotein gene, the VP2 encoding region alone, the mature VP2, and the immunogenic/neutralizing domains of VP2 have been put forth as new candidate vaccines (Gao et al., 2013; Pradhan et al., 2014).

DNA vaccines against IBDV have been developed by encoding VP2 or the polyprotein gene of IBDV. These vaccines often conferred only partial protection (Chen et al., 2011; Hsieh et al., 2010; Li et al., 2003; Rong et al., 2005). Effective vaccine adjuvants need to improve the immunogenicity of non-replicating full antigen, subunit, or DNA vaccines. These vaccine

adjuvants induce a stronger cell-mediated immunity, indicated by higher antigen-specific T cell proliferations as well as an elevated production of the cytokines IL-2 incomparison to vaccinations with the vaccine alone (Kumar et al., 2009). IBDV DNA vaccine efficacy can be improved by coadministering plasmid-encoded chicken interleukin-2 (chIL-2) or CpG-ODN (Hulse and Romero, 2004; Mahmood et al., 2006). VP2-4 DNA+IL-18 vaccine induced a higher level of protection against a challenge compared to vaccinations with the DNA vaccine alone (Gao et al., 2013). A vaccine containing a fusion protein consisting of VP2+chicken IL-2 led to an enhanced immunogenicity in vaccinated chickens in comparison to VP2 vaccination alone (Liu et al., 2005). Vaccination with a DNA-VP2+chicken IL-7 vaccine led to a higher immunogenicity and demonstrated a better protective efficacy (Huo et al., 2016). However, not all recombinant cytokines showed an improvement of the IBDV vaccine response such as the combination with IFNs and IL-1β with an inactivated IBD vaccine (Schijns et al., 2000). Other adjuvant candidates, such as porcine lactoferrin (Hung et al., 2010), HSPs (Maity et al., 2015), chicken beta-defensin-1 (Zhang et al., 2010), and synthetic PAMP mimics such as CpG oligodeoxynucleotides, in combination with different vaccine types, have been experimentally tested as well (Pitcovski et al., 2003; Wang et al., 2003). Vaccination with a C-terminal domain of the HSP70 of *Mycobacterium tuberculosis* in a VP2 DNA vaccine induced higher antibody levels and complete protection against IBDV in comparison to the VP2 DNA vaccine alone (Maity et al., 2015). None of these vaccines are currently available in the market for use.

Even though vaccines play an important role in the control of this disease (Al-Natour et al., 2004 al., 2004), in some cases vaccinations have been considered ineffective in the protection against IBDV infections (Islam et al., 2003). Vaccine transportation, storage, inappropriate diluents, disinfectants, sanitizers (chlorine and glutaraldehyde) in diluents, or use of hot water to reconstitute the vaccine could inactivate the vaccine viruses, which decrease the control of this disease (Prandini et al., 2016). All in all, these risk factors may leave vaccinated birds still susceptible to IBD.

4.2. Gut-associated lymphoid tissue

The gastrointestinal tract (GIT) is an area where the host's infection-susceptible tissues are subjected to close contact with outside agents and pathogens. Gut-associated lymphoid tissues (GALT) are the first line of defense against pathogen invasion from the environment (Liebler-Tenorio and Pabst, 2006). In chickens, the GALT is distributed along the entire intestine (Qureshi et al., 1998). It is a part of the mucosa-associated lymphoid tissue (MALT), consisting of organized tissues with single and/or multiple lymphoid follicles, as well as freely dispersed lamina propria lymphocytes (LPL) (Smith and Beal, 2008). It includes organized lymphoid tissues such as the BF, CT, Peyer's patches (PP), Meckel's diverticulum, and other lymphoid aggregates located within the lamina propria (LP) along the gastrointestinal tract (Befus et al., 1980). Figure 3 provides the location of these lymphoid tissues in the chicken intestinal tract. The GALT is the key immunological system, it is estimated to comprise more immune cells than any other tissue (Kasahara et al., 1994). These associated structures provide a site of stimulation of innate and acquired immune functions through contact with antigens (Friedman et al., 2003; Jeurissen et al., 1993; Shira et al., 2005). One of the key functions of the GALT is to distinguish innocuous antigens from pathogenic microorganisms and to elicit an appropriate response. It provides the conditions necessary to induce an appropriate immune response, such as IgA production by B cells (Shira et al., 2005).

The BF, as the primary lymphoid tissue for B cell development and maturation (Liebler-Tenorio and Pabst, 2006; Reynaud et al., 1991), is located dorsally to the cloaca. It has also been thought of as a secondary lymphoid organ due to the mucosal and submucosal regions of the bursal canal, which display multiple lymphoid follicles (Oláh and Vervelde, 2008). In the bursa, the lymphoid follicles are not in contact with each other. The peripheral cortex is separated from a central medulla by a capillary network and a basal membrane (Dasso et al., 2000; Nagy et al., 2001; Nagy et al., 2004). According to Nagy et al. (2004), the cortex contains a large number of B lymphocytes, and a small number of macrophages and cortical mesenchymal reticular cells (Nagy et al., 2004). The medulla contains a number of

heterogeneous B lymphocytes, some macrophages, and some secretory dendritic cells as well as reticular epithelial cells (Nagy et al., 2004).

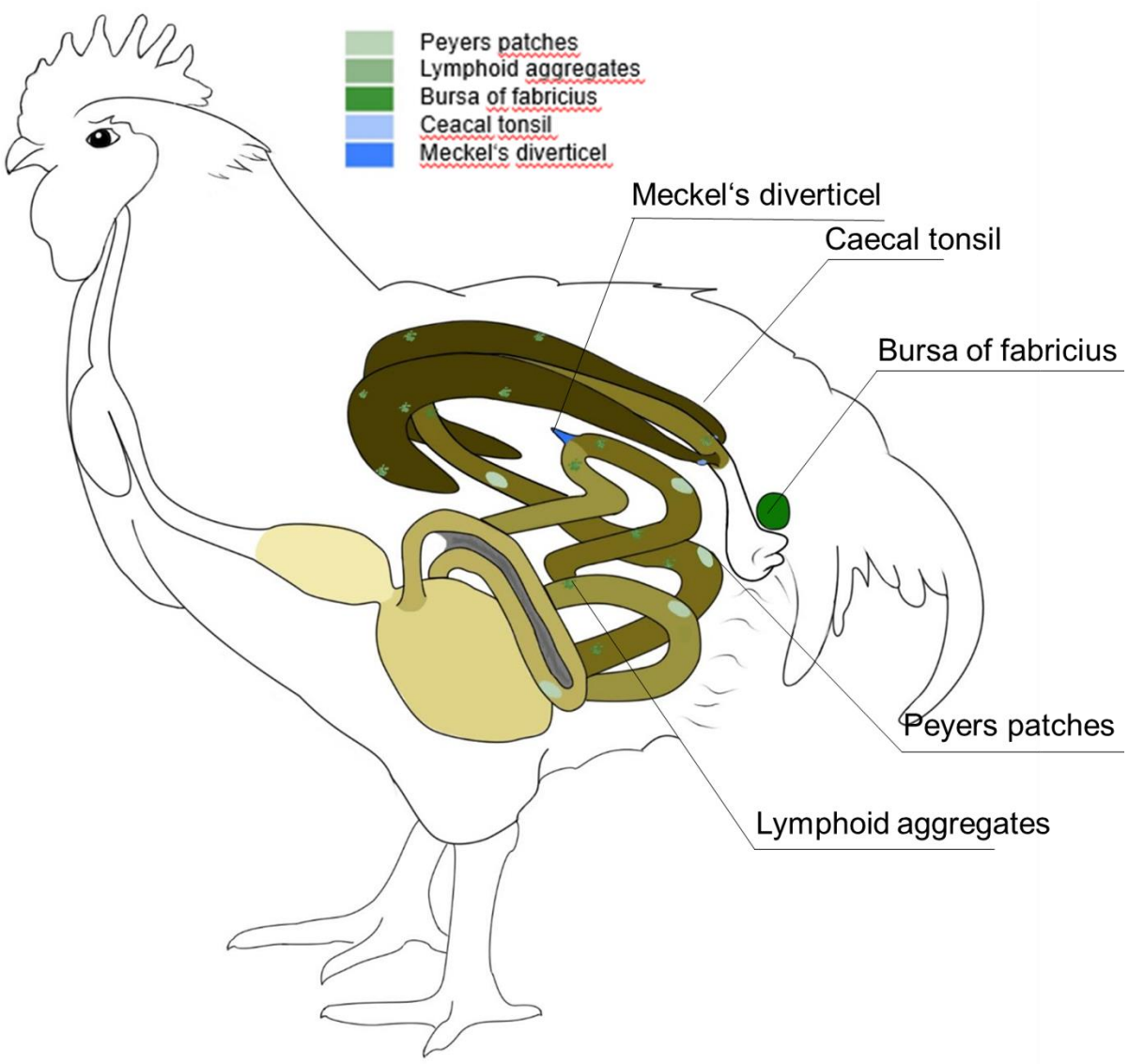

Figure 3: The location of GALT in the chicken intestinal tract (Casteleyn et al., 2010).

The CT are large lymphoid tissues located at the caeco-rectal junction (Del Moral et al., 1998). They produce the precursors of effector immune cells, which are recruited to mucosal surfaces of the intestine (Yurong et al., 2005). CT comprises multiple follicles which are overlaid by a microfold (M) cell-rich epithelium (Kitagawa et al., 2000). These follicles

consist mainly of surface IgG+ and IgM+ B cells and only a small number of IgA+ B cells (Kitagawa et al., 1998; Kitagawa et al., 2000).

The PP are lymphatic aggregates generally dispersed along the epithelium and LP throughout the chicken GALT. The PP contain M cells and follicles with two different zones (a B-cell-dependent subepithelial zone and a T cell-dependent central zone) (Cerutti et al., 2013).

The Meckel's diverticulum is located at the conjunction of jejunum and ileum. It has been suggested to be a lymphoid organ due to the presence of germinal centers (Lillehoj and Trout, 1996). It contains a large number of plasma cells and a small number of granulocytes and monocytes as well as macrophages. No erythrocytes or thrombocytes were observed in Meckel's diverticulum (Oláh and Glick, 1984).

Lymphoid cells and aggregates are present in the epithelium and LP throughout the entire gut. They are considered a lymphoid organ due to the presence of immune cells such as M cells, B and T lymphocytes, dendritic cells, and macrophages.

The intraepithelial lymphocytes (IEL) are also regarded as important parts of the gut immune system (Beagley and Husband, 1998). They are distributed along the entire intestinal epithelium with T, dendritic cells and NK cells. Studies also demonstrated that a number of immune cells such as plasma cells, lymphocytes, macrophages, and granulocytes are present in the LP (Sheridan and Lefrançois, 2010).

4.2.1. *The development of the gut-associated immune system*

The GALT is incomplete at hatch, and it is colonized more rapidly with immune cells than other immune tissues (Schat and Myers, 1991). The development of the GALT is accompanied by rapid physical and functional development of the gastrointestinal tract (Uni, 1999). Enterocytes play an important role during gut development. It has been shown that enterocytes undergo an increase in size and adopt a columnar phenotype, with defined microvillus structures on the luminal face of the gut after hatch (Geyra et al., 2001). The differentiation of enterocytes into mucus–producing goblet cells occurs at hatch, but their number increases rapidly afterwards (Uni et al., 2003). This extensive enterocyte proliferation is attributed to the formation of the basic structures of the gut, such as the establishment of the crypt-villus unit in the small intestine (Geyra et al., 2001; Uni et al., 1999). The development of the BF occurs between eight and fourteen days of embryonic development. Prebursal stem

cells expand extensively to form follicles (Ratcliffe et al., 1986). According to Gasc et al.'s study, a further differentiated BF with a large number of lymphoid cells and some stem cells is present at ED15 (Gasc and Stumpf, 1981). The bursal lymphoid follicles in chickens increase in size and numbers from the time of embryonation until approximately two months post-hatch (Milićević et al., 1986; Sanchez-Refusta et al., 1996). B cells in the bursa begin to migrate from the BF to gut-associated lymphoid tissues at ED18 (Linna and Liden, 1969). B cell development takes place in three stages: prebursal, bursal, and post-bursal (Sayegh et al., 2000). CD79a (also known as Ig-α or mb-1) is an integral membrane protein that is expressed at the very early stages of B cell development, whereas CD79b expression is essential for later stages of B cell development (Sayegh et al., 2000).

The export of bursal B cells and thymic T cells to the periphery is responsible for an increasing number of different lymphoid cell types in the intestinal epithelium and in the specialized lymphoid tissues such as PP and CT (Befus et al., 1980). The size and the cellular complexity of the LP and the IEL compartments increase with age (Befus et al., 1980; Del Moral et al., 1998; Jeurissen et al., 1993). This development of the GALT might partly depend on the presence of enteric microbiota. B cells were detected in CT at hatch, but these cells only express IgM. T cells were also detected in the LP and the epithelium of the gut, but they do not show cytotoxic ability at hatch. According to Teirlynck et al., small aggregations of macrophages and lymphocytes are observed in the gut of two-week-old broilers (Teirlynck et al., 2009). Fully mature lymphoid tissue starts to develop from five to seven weeks of age and remains active until the age of 21 months (Olah et al., 1984; Oláh et al., 2003).

GALT maturation occurs probably in two stages (Fagerland and Arp, 1992; Stanley et al., 2012b). The primary stage begins during the first week post-hatch. At around four days post-hatch, B cells start to populate the distal parts of the intestine, with an increase in the expression of IL2 and IFNγ. T cells start to populate during the primary stage. A substantial increase in the number of CD3+ cells was observed in all gut segments. This increase in CD3 mRNA expression levels can be attributed to gut colonization by NK and T cells (Bar-Shira et al., 2003). During the second stage in the second week of life, a further increase in CD3+ cell numbers in the gut and a continued upregulation of IL2 and IFNγ mRNA expression levels were shown (Sayegh et al., 2000).

The development of the GALT is associated with successive waves of γδ and αβ T cells derived from the thymus (Dunon et al., 1997). During the development of the bird, the T cell composition of the GALT changes, such as the numbers of IEL T cells expressing T cell receptor (TCR) 1 (γ/δ) increase compared to TCR 2 (α/β1) positive cells (Lillehoj and Chung, 1992).

4.3. The microbiota of chickens

A diverse microbial community comprised of bacteria, yeasts, archaea, ciliated protozoa, anaerobic fungi, and bacteriophages has been observed in the intestinal tract of chickens (Mackie, 2002). Previous studies demonstrated that the digestive tract of a newly hatched chicken is sterile and that microbiota colonization begins through contact with the environment (Crhanova et al., 2011). However, Binek et al. observed that bacteria can already be found in the caecum of unhatched embryos (Binek et al., 2000). A recent study demonstrated the presence of bacteria in the caecum, liver, and yolk sac at ED 18 and 20 (Kizerwetter-Świda and Binek, 2008). The normal intestinal microbiota in the small intestine, caecum, and large intestine of chickens develops after hatching (Macpherson and Harris, 2004). Subsequently, the complexity of the gut microbiota gradually increases, with the most dramatic developments taking place during the first week of life. The gut microbiota community establishes itself in the small intestine within the first two weeks post-hatch (Blakey et al., 1982; Engberg et al., 2000). At 40 days post-hatch, the microbiota composition becomes stable (Lan et al., 2005). Recent studies estimate that the GI tract of a broiler chicken is colonized by over 600 species of bacteria from over 100 different genera (Apajalahti et al., 2004; Torok et al., 2011). The most abundant phylum in the intestinal microbiota is *Firmicutes*, followed by two minor phyla, *Proteobacteria* and *Bacteroidetes*. It was also demonstrated that other members of phyla such as *Actinobacteria*, *Tenericutes*, *Cyanobacteria*, and *Fusobacteria* can be found in low numbers in the chicken gut (Qu et al., 2008; Waite and Taylor, 2014). Figure 4 provides the basic structure of the gut microbiota in the intestine.

The GIT of chickens comprises the esophagus, crop, proventriculus, gizzard, duodenum, jejunum, ileum, caecum, colon, and cloaca.

The density of bacteria in the crop can reach from 10^8 to 10^9 bacteria cells/g. It consists mainly of *Lactobacillus* (Gong et al., 2007; Sekelja et al., 2012). Other genera such as

Clostridiaceae, Bifidobacterium, Enterobacteriaceae, Enterococcus, or *Enterobacter* can be found in the crop as well (Rehman et al., 2007; Sekelja et al., 2012). *Lactobacillus* species such as *L. frumenti, L. antri, L. mucosae, L. acidophilus, L. reuteri, L crispatus, L. salivarius, L. fermentum, L. amylovorus, L. aviarius, L. johnsonii and L. gallinarum* have been observed (Fuller, 1973; Rehman et al., 2007).

Little is known about the composition of bacteria in the proventriculus. The density of bacteria in the proventriculus is about 10^4 to 10^6 bacteria cells/g, and *Lactobacilli* has been shown to dominate (Engberg et al., 2004). It was demonstrated that a small number of *Enterococci* and *Escherichia* are also found in the proventriculus (Engberg et al., 2004).

In the gizzard, the density of bacteria spans 10^5 to 10^7 bacteria cells/g. It is dominated by *Lactobacillus* and *Clostridiaceae*. *Enterococcus*, *Campylobacter*, and *Escherichia* are also isolated from the gizzard (Fuller, 1973; Rehman et al., 2007; Sekelja et al., 2012).

Lactobacillus is the most abundant genus in the duodenum. Salanitro et al. also found *Streptococcus, Escherichia*, and *Eubacterium* in the duodenum. *Lactobacillus* species such as *L. aviaries* and *L. salivarius* are isolated from the duodenum (Gong et al., 2007; Lu et al., 2003a; Salanitro et al., 1974).

The jejunum is dominated by *Lactobacillus* species such as *L. aviaries, L. salivarius, L. crispatus, L. johnsonii*, and *L. reuteri* (Gong et al. 2007; Stanley et al. 2012a). Genera such as *Candidatus Arthromitus, Clostridium, Ruminococcus*, *Escherichia, Enterococcus*, and *Enterobacteria* were isolated in a number of studies (Gong et al., 2007; Stanley et al., 2012a).

The ileum is dominated by *Lactobacillus*, followed by *Enterococcus, Streptococcus, Coliforms, Candidatus Arthromitus, Escherichia*, and *Clostridium* (Gong et al., 2002b; Lu et al., 2003a; Lu et al., 2003b; Pourabedin et al., 2015; Salanitro et al., 1974; Shaufi et al., 2015; Van der Hoeven-Hangoor et al., 2013). In more detail, *Lactobacillus* species such as *L. salivarius, L. delbrueckii, L. acidophilus, and L. crispatus* are isolated from the ileum *(*Lu et al. 2003).

Figure 4: A map of the gastrointestinal tract with major taxa. Data on taxa is modified from (Gong et al., 2007; Qu et al., 2008; Saengkerdsub et al., 2007; Yeoman et al., 2012) and present phyla (bold) and genera.

The bacterial community in the caecum varies in different studies. The density of bacteria can reach up to 10^{11} bacteria cells/g. According to Gong et al. (2007) the caecum is mainly dominated by *Clostridium*; the genera *Lactobacillus* and *Ruminococcus* are also found in the caecum (Gong et al., 2007). Other genera such as *Eubacterium, Faecalibacterium, Blautia, Butyrivibrio, Hespillia, Megamonas, Veillonella*, *Anaerostipes*, and *Escherichia* were also found in several studies (Gong et al., 2002a; Lu et al., 2003a; Wei et al., 2013; Zhu et al., 2002). Danzeisen et al.(2011) showed that the majority of *Clostridia*, which were detected in

the caecum, fall primarily into three main families: *Clostridiaceae*, *Lachnospiraceae* and *Ruminococcaceae* (Danzeisen et al., 2011). Moreover, Yin et al. (2010) found that *Enterococcaceae*, *Enterobacteriaceae*, and *Bacteroidaceae* are other abundant families in the caecal microbiota (Yin et al., 2010). Xiao et al. (2016) indicated that *Bacteroides* may also be the dominant genus in the caecum in comparison to other intestine sections (Xiao et al., 2016). The caecum is also rich in unknown and unclassified bacterial residents. At the species level, *Bacteroides fragilis, L. crispatus, L. johnsonii, L. salivarius*, and *L. reuteri* together comprise more than 40% of caecal microbiota (Stanley et al., 2012a).

In the cloaca, the microbiota composition fluctuates greatly, depending on varying contributions of microbiota from different GI segments (Sekelja et al., 2012). *Lactobacillus, Clostridium, Fecalibacterium, Ruminococcus, Escherichia, Bacillus, Eubacterium, and Fusobacterium* are present in the cloaca (Sekelja et al., 2012; Zoetendal et al., 1998). Videnska et al. (2014) demonstrated that the common families are *Lactobacillaceae*, *Peptostreptococcaceae, Streptococcaceae*, *Clostridiaceae,* and *Enterobacteriaceae* (Videnska et al., 2014).

4.3.1. *Factors influencing the gut microbiota composition*

It was demonstrated that the composition of the microbiota is influenced by the host (such as gender, age, genotype, maternal component effects), environment (antibiotics, pathogens), and diet factors (feed composition and feeding strategy) (Table 7) (Barnes et al., 1980; Kizerwetter-Świda and Binek, 2008; Xiao et al., 2016).

In young birds, the diversity of the microbiota composition increases quickly as the birds age (Awad et al., 2016b).

The influence of gender has an impact on the gut microbiota composition in males and females (Lumpkins et al., 2008). It was demonstrated that female chickens had clearer separation in distribution than male birds (Zhao et al., 2013). Studies indicated that 11 species, which beyond to *Lactobacillus*, were affected by gender in both high weight and low weight lines of chickens. The gut microbiota composition is also modified by the chicken's genetic background. According to Wielen et al. (2002)'s study, individual chickens have their own unique microbiota (Wielen et al., 2002). The genetic background of a chicken may affect its microbiota composition, either directly through secretions into the gut, control of gut

motility, and modification of epithelial cell surfaces, or indirectly, through food and lifestyle preferences (Zhao et al., 2013).

Dietary compounds modify gut microbiota composition. It was demonstrated that it is affected through dietary changes (Degnan and Macfarlane, 1991; Macfarlane et al., 1998; Mead, 1989). Analysis of GC percentage showed that a wheat-based diet increased bacterial species displaying 50%-55% GC content, and suppressed those with a GC content of 60%-69% in chicken (Apajalahti et al., 2004). An increase in the dietary fat source (soy oil or a mixture of lard and tallow) led to a lower abundance of *Lactobacilli* and *Clostridium perfringens* in the ileum of broiler chickens (Józefiak et al., 2016; Knarreborg et al., 2002).

Antibiotics provided through feed modified the gut microbiota composition and were shown to reduce the stability of the microbiota composition by the modification of the abundance of *Lactobacillus* in the chickens' intestines (Lan et al., 2005). Gong et al. showed only 31% similarity in the composition of the microbiota in the caecum between antibiotic treated and non-treated chickens (Gong et al., 2008).

Major stresses are also considered to be factors that might modify the gut microbiota composition. It is commonly accepted that the health status of birds plays a role in the modulation of the intestinal microbiota composition. The immunity of the host and integrity of the intestinal barrier prevent bacteria from the intestinal tract from entering into the blood stream or invading organs (Guarner and Malagelada, 2003; Marchiando et al., 2010). Once the barrier is damaged, opportunistic pathogens can invade tissues and cause infections.

Table 7: Factors influencing the gut microbiota composition

Factor	Critical findings	Reference
gender	male and female chickens showed a different gut microbiota composition	(Lumpkins et al., 2008)
	females had a clearer separation in comparison to males birds	(Zhao et al., 2013)
	an individual chicken has its own and unique intestinal microbial composition	(Wielen et al., 2002)
age	one-day-old birds had more *Proteobacteria*, *Firmicutes*, and *Tenericutes* as well as a lower diversity within the bacterial communities compared to 28 day old birds	(Awad et al., 2016b)
	the abundance of *Enterobacteriales* decreased with age ↑*Clostridiales* and *Lactobacillales* in three-week-old chicks compared to one day old chicks	(Juricova et al., 2013)
diet	dietary fat source (soy oil or a mixture of lard and tallow) ↓ *Lactobacilli* and *Clostridium (C.) perfringens* in the ileum	(Knarreborg et al., 2002)
	non-starch polysaccharides ↑ *C. perfringens*	(Choct et al., 1996)
	sorghum-based diet ↓ anaerobic bacteria and *Lactobacilli*	(Shakouri et al., 2009)
	enzyme supplement ↓ lactose-negative *Enterobacteria*	(Shakouri et al., 2009)
	the microbiota composition was more diverse with rye-based diets than that of birds fed on barley-based diets	(Jozefiak et al., 2010)
	dietary enzymes (xylanase and β-glucanase) ↑ intestinal lactic acid bacteria and ↓ the population of adverse and pathogenic bacteria such as *E. coli*	(Rodríguez et al., 2012)
	essential oils (thymol, carvacrol, eugenol, curcumin, and piperin) ↓ *C. perfringens colonization*	(Mitsch et al., 2004)
antibiotic	only 31% similarity of microbiota composition was observed in the caecum between antibiotic treated and non-treated chickens	(Gong et al., 2008)
	↓ the stability of the microbiota composition by the modification of the abundance of *Lactobacillus*	(Lan et al., 2005)
	avilamycin, bacitracin methylene disalicylate and enramycin modify the composition of the intestinal bacterial community of birds	(Pedroso et al., 2006)
pathogen	Eimeria induced ↓ *Ruminococcaceae* and ↑ three unknown *Clostridium* species	(Wu et al., 2014)
	Eimeria induced ↑ an unclassified order of *Mollicutes*	(Stanley et al., 2012b)

table 7: continuing

pathogen	*Salmonella* enterica induced ↑*Enterobacteriales*, ↓ *Clostridiales*, *Lactobacillales*, and *Bifidobacteriales*.	(Juricova et al., 2013)
	C. jejuni colonization induced ↓ *Escherichia coli* and ↑ *Clostridium* spp.	(Awad et al., 2016b)
	C. jejuni colonization modified the caecal beta-diversity, ↑*Bifidobacterium* and affected *Clostridia* and *Mollicutes*	(Thibodeau et al., 2015)
	MDV ↑ *Lactobacillus* and *Gammaproteobacteria*	(Perumbakkam et al., 2014)

MDV=Marek's disease virus; ↑=upregulation; ↓=downregulation; *C. jejuni*=*Campylobacter jejuni*.

4.4. *Campylobacter jejuni (C. jejuni)*

C. jejuni has been considered as one of the most common bacterial causes of human gastroenteritis in the industrialized world. To date, this bacterium poses a serious health burden in industrial countries (Dasti et al., 2010a). In 2011, a total of 50.3 cases per 100,000 inhabitants or 220,209 confirmed cases were investigated from humans throughout the European Union (EFSA, 2013; EFSA, 2011). There is overwhelming evidence that consumption of contaminated broiler chicken and turkey meat presents a predominant source of *Campylobacter* transmission to humans. Because the intestine of living poultry is the only niche where amplification of *C. jejuni* can occur throughout the food chain, control of *C. jejuni* colonization or shedding by broilers, and subsequently external *C. jejuni* contamination of broilers during rearing, would have a great impact on human campylobacteriosis incidence, as less *C. jejuni* would reach consumers. In poultry, the definition of pathogenesis of *C. jejuni* in chickens is controversial. There is an increasing number of publications demonstrating that *C. jejuni* could induce mild cell-mediated immune responses, including infiltration of T cell subpopulations as well as cytokine production in the chicken gut, in some cases, this bacteria could induce gut lesions as well as clinical symptoms (Han et al., 2016a; Humphrey et al., 2014). Investigations indicated that the immune status of chickens could affect the pathogenesis of *C. jejuni*. One recent study demonstrated that immunosuppressed chickens showed higher frequency of invasion of *C. jejuni* (Vaezirad et al., 2016). Immunosuppressive viruses, especially IBDV, may affect the pathogenesis of *C. jejuni*. Since the effect of immunosuppression on immunity might in turn affect the development of gut microbiota composition, it might subsequently influence the colonization pattern of *C. jejuni*. This has not been closely studied, but it is important to investigate.

4.4.1. *Etiology*

The genus *Campylobacter* belongs to the family *Campylobacteraceae*, the order *Campylobacterales*, and the phylum *Proteobacteria*. At present, the genus *Campylobacter* includes 25 validated species and 8 subspecies (Man, 2011). *Campylobacter* spp. are gram negative, slender, and spirally curved rods. The bacterium size is 0.2 to 0.8 µm wide and 0.5 to 5 µm long (Brenner and Farmer, 1984). The genome of *C. jejuni* has a size of about 1.6 Mbp with a GC content of 30%, the percentage coding of the bacterial DNA is approximately 90% (Man, 2011).

4.4.2. *Campylobacter in poultry*

C. jejuni has been regarded as a commensal microbe in chickens. Poultry has been considered a major reservoir for *Campylobacter*. Studies have demonstrated that commercial poultry such as chickens, turkeys, and ducks as well as wild birds can be colonized by *Campylobacter* without exhibiting any clinical symptoms during experimental infection (Yogasundram et al., 1989). Compared to wild birds, domestic flocks show higher rates of *Campylobacter* colonization (Dasti et al., 2010b). *C. jejuni* mainly colonizes the chicken caecum and is located in the deep caecal crypts. Its primary habitat is the mucus layer near the epithelium (Lee and Newell, 2006). However, recent studies suggest that *C. jejuni* may not always be a commensal, but shows pathogenic characteristics in the chicken gut (Smith et al., 2008). Humphrey et al. (2014) demonstrated that *C. jejuni* induced diarrhea, inflammation, and mucosal damage in the gut of rapid-growing boiler chickens (Humphrey et al., 2014). *In vitro* investigations also showed that *C. jejuni* could invade and evade crypt epithelial cells of chickens and induce mild inflammatory responses, as well as necrosis or apoptosis (Van Deun et al., 2008b). *Campylobacter* infections strongly interfere with Ca2+ signaling (Awad et al., 2015). *C. jejuni* inoculation affected the gut barrier functions by inducing fluid and electrolyte secretion, accompanied by an inflammatory response (Berkes et al., 2003). This bacterium also modifies the gut microbiota composition with a decrease in propionate, isovalerate, and isobutyrate producing bacteria in the chicken gut (Awad et al., 2016a).

4.4.3. *Factors affecting the pathogenesis of C. jejuni*

Many factors affect *C. jejuni* colonization in chickens, including MDA, age, *C. jejuni* strain, infecting dose, and microbiota, as well as feeding strategy (Han et al., 2016a; Han et al., 2016b; Stas et al., 1999). As table 8 indicates findings may vary and are even controversial for the different factors.

Studies demonstrated that different *C. jejuni* strains have different colonization abilities in chickens (Hepworth et al., 2011; Shanker et al., 1990). A strain-to-strain variation study in broilers showed different infection strategies of *C. jejuni* within chickens, including invasiveness as well as colonization of the caecum (Chaloner et al., 2014). For example, the *C. jejuni* M1 strain showed a rapid colonization, concentrated mainly in the ceca, while the *C. jejuni* 13126 strain showed weaker colonization of the caeca and preferred to colonize the

upper GI tract instead. Moreover, this strain showed a higher potential for extra-intestinal spread compared to the *C. jejuni* M1 strain (Chaloner et al., 2014).

The role of MDA on the colonization of *C. jejuni* in chickens is still a controversial issue. No clinical signs were observed in three-day-old chicks, while diarrhea was observed in hatchlings at 12 hours post-hatch after oral inoculation at a dosage of 10^8 colony forming units (CFU) (Welkos, 1984). Three-day-old layer pullets, which had *C. jejuni*-specific MDA, showed a lower number of colonized birds compared to MDA negative SPF layer pullets (Sahin et al., 2003). Three-day-old MDA positive broilers and three-week-old MDA negative broilers were also compared. Data demonstrated that *C. jejuni* shedding occurred earlier in birds inoculated at three weeks of age compared to birds inoculated at three days of age (Sahin et al., 2003). Conflicting results have demonstrated that newly hatched birds, which showed highest levels of *C. jejuni*-specific MDA, were as susceptible to *C. jejuni* as three-week-old birds (Cawthraw and Newell, 2010b). One recent study showed that chickens inoculated with *C. jejuni* at 22 days post-hatch showed a lower colonization rate compared to one-, 10-, and 31-day-old chickens (Han et al., 2016a).

The effect of dosage on the colonization pattern of *C. jejuni* has been clearly demonstrated. Chickens inoculated with higher doses (10^7 and 10^9 CFU) showed higher frequencies of enteritis and an earlier onset of disease in comparison to chickens inoculated with lower doses (10^1 to 10^5 CFU) (Welkos, 1984). SPF chickens given oral inoculation at lower doses (10^1 CFU) showed a low or zero colonization rate (Ahmed et al., 2002; Jones et al., 2004). Two-fold higher doses were required for successful colonization of two-week-old birds compared to one-day-old birds (Knudsen et al., 2006).

Genetic background has an impact on the colonization of *C. jejuni*. Two-to three-day old White Leghorn chickens were less susceptible to *C. jejuni*-induced diarrhea than Starbro birds (Sanyal et al., 1984). Two different broiler lines showed a different level of resistance to *C. jejuni* colonization (Humphrey et al., 2014; Li et al., 2010b). Moreover, Humphrey et al. (2014) demonstrated that *Campylobacter* particularly affected rapidly-growing broilers and induced more lesions and a stronger inflammatory as well as a lower regulatory immune response in comparison to slow-growing broiler chickens (Humphrey et al., 2014). One recent study also indicated that layer type and broiler type chickens show different susceptibilities to *C. jejuni* colonization (Han et al., 2016b).

Table 8: Factors affecting the pathogenesis of *C. jejuni* in poultry

Factor	Critical findings	Reference
MDA	presence and protective effect of MDA against *C. jejuni*	(Cawthraw and Newell, 2010a)
	do not protect against *C. jejuni*	(Han et al., 2016a)
	protective role of MDA against *C. jejuni* in young birds	(Sahin et al., 2003)
strain	strain variations in *C. jejuni* infection ecology	(Chaloner et al., 2014)
	pathogenesis of different *C. jejuni* isolates	(Dhillon et al., 2006)
	different strains showed different colonization patterns of the caecum in chickens.	(Hänel et al., 2009)
genetic background	*C. jejuni* colonization is not affected by the bird growth rate and breed	(Gormley et al. , 2014)
	fast-and slow-growing broilers showed different inflammatory responses after *C. jejuni* infections	(Williams et al., 2013)
	the *C. jejuni*-induced caecal transcriptome differed in two genetic lines	(Li et al., 2011)
	two genetic lines differed in caecal *C. jejuni*-colonization	(Li et al., 2010a)
	two to three day old White Leghorn chickens were less susceptible for *C.jejuni* than birds from the Starbro strain.	(Sanyal et al., 1984)
	C. jejuni induced changes of the intestinal flora composition	(Sofka et al., 2015)
	broilers were colonized to a higher extend and the local immune cell response was different compared to the SPF –layer type birds	(Pielsticker et al., 2016)
	C. jejuni induce a more vigorous immune response in BT birds compared to LT ones	(Han et al., 2016b)
gut microbiota	live *Enterococcus faecalis (E. faecalis)* reduced *C. jejuni in vitro* but not *in vivo*	(Robyn et al., 2012)
	E. faecalis MB 5259 inhibited *C. jejuni* MB 4185 growth, no inhibition was observed in the in vivo experiments independent of the inoculum size	(Wang et al., 2014)
food additives	bacteriocins reduced *C. jejuni* colonization and altered gut morphology	(Cole et al., 2006)
	effect of caprylic acid in the reduction of *C. jejuni* colonization	(Hovorková and Skřivanová, 2015)
	probiotic isolates reduced *C. jejuni* colonization	(Arsi et al., 2015)

table 8: continuing

food additives	feed additives (OA and botanicals in a lipid embedding matrix) reduced the amount of caecal *C. jejuni* in slaughter age broilers	(Grilli et al., 2013)
	effect of formic acid in the reduction of *C. jejuni*	(Skånseng et al., 2010)
	MCFA feed supplementation reduced *C. jejuni* colonization	(van Gerwe et al., 2009)
	SCFA and L-lactate controlled *C. jejuni* colonization	(Van Deun et al., 2008b)
	diet composition modified the concentration of SCFA and altered *C. jejuni* colonization	(Molnar et al., 2015)
	WBES diet induced significant lower CFU of *C. jejuni*	(Van Deun et al., 2008b)

SCFA=short-chain fatty acid; MCFA=medium-chain fatty acid;

WBES=wheat-based diet with non-starch polysaccharide-degrading enzyme supplementation

Feeding strategies were shown to affect the colonization of *C. jejuni* in a number of studies. Chickens fed with protein derived from plants had significantly lower CFU numbers of *C. jejuni* in comparison to birds fed with an animal-protein-based feed (Udayamputhoor et al., 2003). A diet rich in oat/barley hulls delayed the horizontal spread of *C. jejuni* in broilers (Moen et al., 2012). Feed additives, such as microencapsulated propionic acids and sorbic acids (Grilli et al., 2013), or various combinations of formic acid and sorbate (Skånseng et al., 2010), could reduce the caecal CFU numbers of *C. jejuni* in broilers. Medium chain fatty acid feed supplementation reduced the probability of *C. jejuni* colonization in broilers (Van Gerwe et al., 2010). Overall, the diet composition for broiler chickens modifies the colonization dynamics of *C. jejuni* (Molnar et al., 2015). Recently, feed additive studies have been conducted to find an additive that will reduce *C. jejuni* colonization in chickens. It has been shown that feed additives with medium chain fatty acids (MCFA) may reduce *C. jejuni* colonization in broilers (Hovorková et al., 2015; Van Gerwe et al., 2010).

4.4.4. *Influence of C. jejuni on gut microbiota*

Studies showed that *C. jejuni* had an impact on the gut microbiota (Sofka et al., 2015; Thibodeau et al., 2015). *C. jejuni* colonization in chickens was associated with a lower

abundance of *Lactobacillus* and *Corynebacterium,* and with a higher abundance of *Streptococcus* and *Ruminococcaceae* (Kaakoush et al., 2014). A lower abundance of *Firmicutes* and higher abundances of *Proteobacteria* as well as *Bacteriodetes* was observed in *C. jejuni*-inoculated chickens compared to *C. jejuni*-free chickens (Sofka et al., 2015). Awad et al. (2016b) demonstrated that *C. jejuni* colonization modified the gut microbiota composition by decreasing the abundance of *Escherichia coli* at different gut sites in *C. jejuni*-inoculated chickens (Awad et al., 2016b). *C. jejuni* may modify the gut microbiota composition due to the utilization of *s*hort-chain fatty acids (SCFAs) as a carbon source (Awad et al., 2016a; Masanta et al., 2013). Previously Van Deun et al. (2008a) indicated that SCFA, such as butyrate acetate and propionate, play a role in controlling *C. jejuni* colonization (Van Deun et al., 2008a).

4.4.5. *Relationship between C. jejuni and other immunosuppressive pathogens*

Another factor which might influence *C. jejuni* colonization patterns is the immune status of the host. It was shown that coinfection with IBDV leads to a higher rate of *C. jejuni* colonization in birds than mono-infection with *C. jejuni* (Subler et al., 2006). Furthermore, chickens vaccinated with an IBDV live vaccine exhibited lesions in the liver and intestine and had low *C. jejuni* antibodies in comparison to *C. jejuni* mono-inoculated chickens (Stojanov et al., 2008). One recent study demonstrated that *C. jejuni* antibody production plays a role in the clearance of intestinal infection of *C. jejuni*. Bursectomized birds showed a failure to clear *C. jejuni* in the caecum (Lacharme-Lora et al., 2017). Open questions about the mechanism remain.

5. Goals and objective

The goal of this study was to understand the effect of vvIDBV on local gut-associated immunity and gut microbiota composition. We speculate that IBDV modifies the local gut immunity, especially the humoral and subsequently the microbiota composition, which may lead to a change in the colonization characteristics of *C. jejuni*, and an exacerbation of the infection.

The objectives of the proposed project are:

To determine the effect of early infection with vvIBDV on the development of bursa of Fabricius (BF), caecal tonsils (CT), caecum and the composition of the gut microbiota. The following parameters were compared between IBDV-inoculated and virus-free chickens:

a) Virus antigen load
b) Lesion development
c) Immune responses, including immune cells
d) Humoral immunity
e) Gut microbiota composition

To investigate the effects of IBDV-induced immunosuppression on *Campylobacter jejuni* colonization and pathogenesis. The following parameters were investigated:

a) IBDV antigen load
b) *C. jejuni* colonization rates
c) Gut and bursal lesion development
d) Immune responses, including immune cells and cytokines
e) Humoral immunity
f) Gut microbiota composition

6. INFECTIOUS BURSAL DISEASE VIRUS INFECTION LEADS TO CHANGES IN THE GUT ASSOCIATED-LYMPHOID TISSUE AND THE MICROBIOTA COMPOSITION

Li Li[a], Tereza Kubasová [b], Ivan Rychlik[b], Frederic J. Hoerr[c], Silke Rautenschlein[a]*

a University of Veterinary Medicine Hannover, Clinic for Poultry, Bünteweg 17, Hannover, Germany[a];

b Veterinary Reserch Institute, Hudcova 70, 621 00 Brno, Czech Republic[b]

c Veterinary Diagnostic Pathology, LLC 638 South Fort Valley Road, Fort Valley, Virginia 22652 USA

*Corresponding author: Silke Rautenschlein

Clinic for Poultry

University of Veterinary Medicine Hannover

Bünteweg 17

30559 Hannover, Germany

Tel: 49-511-953-8779

Fax: 49-511-953-8580

Email: Silke.Rautenschlein@tiho-hannover.de

State of publication: publication

PloS one 13(2): e0192066.

Available: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0192066

Authors contributions:

LL and SR conceived and designed the experiments; LL performed the experiments; IR conducted the illuminal sequencing; FJH evaluated the mucosal thickness; LL analyzed the data; LL and SR wrote or helped to draft the paper. All authors read and approved the final manuscript.

The extent of contribution from Li Li to this article:

Scientific design: 40 %

Laboratory work: 90 %

Evaluation: 90 %

Scientific writing: 60 %

6.1. Abstract

Infectious bursal disease (IBD) is an acute, highly contagious and immunosuppressive poultry disease. IBD virus (IBDV) is the causative agent, which may lead to high morbidity and mortality rates in susceptible birds. IBDV-pathogenesis studies have focused mainly on primary lymphoid organs. It is not known if IBDV infection may modify the development of the gut associated lymphoid tissues (GALT) as well as the microbiota composition. The aim of the present study was to investigate the effects of IBDV-infection on the bursa of Fabricius (BF), caecal tonsils (CT) and caecum, and to determine the effects on the gut microbiota composition in the caecum. Commercial broiler chickens were inoculated with a very virulent (vv) strain of IBDV at 14 (Experiment 2) or 15 (Experiment 1) days post hatch (dph). Virus replication, lesion development, immune parameters including numbers of T and B-lymphocytes, macrophage, as well as the gut microbiota composition were compared between groups. Rapid IBDV-replication was detected in the BF, CT and caecum, and accompanied with histological lesions including an infiltration of heterophils and a significant reduction in the total mucosal thickness of the caecum were observed in vvIBDV-infected birds. vvIBDV infection led to a significant increase in T lymphocyte number and macrophages, and a decrease in the number of B lymphocytes compared to virus-free controls. Ilumina sequencing analysis indicated that vvIBDV infection also led to changes in the abundance of *Clostridium XIVa* and *Faecalibacterium* over time. Overall, our results suggested that vvIBDV infection had a significant impact on the GALT and led to a modulation of gut microbiota composition, which may led to a higher susceptibility of affected birds for pathogens invading through the gut.

Keywords:

Infectious bursal disease virus, gut-associated lymphoid tissues, immune cells, gut microbiota composition.

Acknowledgement

This publication was supported by Deutsche Forschungsgemeinschaft and University of Veterinary Medicine Hannover, Foundation within the funding programme Open Access Publishing.

We would like to thank Sonja Bernhardt and Christine Haase for their excellent technical assistance and support. In addition we would like to thank Marina and Thomas for help with the necropsy work. We also would like to thank August C. Hoerr for morphometric assessment of the cecum mucosal. Li Li was supported by the Chinese Scholar Council and Ivan Rychlik was partially supported by AdmireVet project CZ.1.05/2.1.00/01.0006 – ED0006/01/01 from the Czech Ministry of Education.

6. INFECTIOUS BURSAL DISEASE VIRUS INFECTION LEADS TO CHANGES IN THE GUT ASSOCIATED-LYMPHOID TISSUE AND THE MICROBIOTA COMPOSITION

7. INFECTIOUS BURSAL DISEASE VIRUS INOCULATION MODIFIES *CAMPYLOBACTER JEJUNI*-HOST INTERACTION IN BROILERS

Li Li[a], Colin Pielsticker[a], Zifeng Han[a], Tereza Kubasová[b], Ivan Rychlik[b], Bernd Kaspers[c], Silke Rautenschlein[a]*

a University of Veterinary Medicine Hannover, Clinic for Poultry, Bünteweg 17 Hannover, Germany[a];

b Veternary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic[b]

c Department for Veterinary Sciences, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität München, Veterinastr. 13, 80539 Munich, Germany[c].

*Corresponding author: Silke Rautenschlein

Clinic for Poultry

University of Veterinary Medicine Hannover

Bünteweg 17

30559 Hannover

Germany

Tel: 49-511-953-8779

Fax: 49-511-953-8580

Email: Silke.Rautenschlein@tiho-hannover.de

State of publication: publication

Gut Path 10(1): 13.

Available : http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0192066

Authors contributions:

CP and SR conceived and designed the experiments; LL, CP, ZH performed the experiments; TK, IR conducted the illunimal sequencing; BK performed the qRT-PCR analysis of IgA mRNA expression. LL analyzed the data; LL and SR wrote or helped to draft the paper. All authors read and approved the final manuscript.

The extent of contribution from Li Li to this article:

Scientific design: 20 %

Laboratory work: 90 %

Evaluation: 90 %

Scientific writing: 50 %

7.1. Abstract

Campylobacter jejuni (C.jejuni) is considered as a chicken commensal. The gut microbiota and the immune status of host may affect its colonization. Infectious bursal disease virus (IBDV) is an immunosuppressive virus of chickens, which allows secondary pathogens to invade or exacerbate their pathogenesis. To investigate the effect of IBDV-induced immunosuppression on the pathogenesis of *C. jejuni*, broiler chickens were inoculated with a very virulent (vv) strain of IBDV at 14 days post hatch followed by *C. jejuni* inoculation at seven (experiment A) or nine (experiment B) days post IBDV infection. The *C. jejuni*-colonization pattern was comparable between mono-inoculated groups of both experiments, but it varied for vvIBDV + *C. jejuni* co-inoculated groups. The timing between viral and bacterial infection might affect the outcome of *C. jejuni* colonization differently. In experiment A significant higher numbers of colony forming units (CFU) of *C. jejuni* were detected in caecum of co-inoculated compared to *C. jejuni*-mono-inoculated birds in the early phase post bacterial inoculation (pbi). In experiment B the clearance phase was affected in the co-inoculated group with significantly higher CFU at 21 days pbi ($P < 0.05$). vvIBDV-infection led to a depression in lamina propria B cell numbers, total bursal IgA-mRNA expression, and the anti-*C. jejuni*-antibody response starting at 14 days pbi. Both pathogens affected the microbiota composition. Overall, we speculate that humoral immunity may play an important role especially during the bacterial clearance phase. No major differences were seen in local T lymphocyte populations between *C. jejuni*-inoculated groups. Interestingly, both pathogens affected the microbiota composition.

Key words

Campylobacter jejuni; infectious bursal disease virus; immunosuppression; immune response; gut microbiota composition

7.2. Acknowledgement

This publication was supported by Deutsche Forschungsgemeinschaft and University of Veterinary Medicine Hannover, Foundation within the funding programme Open Access Publishing.

Author would like to thank Zifeng Han, Marina Dorber for their excellent technical support and discussion. Li Li was supported by the Chinese Scholar Council and Ivan Rychlik was partially supported by AdmireVet project CZ.1.05/2.1.00/01.0006 – ED0006/01/01 from the Czech Ministry of Education.

8. Discussion

It was hypothesised that IBDV as an immunosuppressive virus of young chickens might not only modify the GALT but also the gut microbiota composition. These changes may influence the defense mechanism of the host allowing secondary pathogens to overcome and invade the gastrointestinal barrier more easily. The first aim of this thesis was to investigate the effect of vvIBDV on the GALT as well as on the gut microbiota composition. To address this aim, we conducted two studies. In study 1, two animal trials were included. Broiler chickens were inoculated with vvIBDV at 14 (Experiment 2) or 15 (Experiment 1) dph, when the MDA were below the breakthrough titer of the virus. We detected viral replication, lesion development, different immune parameters, as well as the gut microbiota composition in the caecum. Virus replication was observed in the GALT including the BF, CT and caecum. vvIBDV infection led to a reduction of caecal total mucosal thickness. A significant higher number of CD4+ and CD8ß+ LPL and a significant decrease in the number of LP B lymphocytes were observed in the caecum of vvIBDV inoculated birds compared to virus-free controls ($P < 0.05$). In addition, vvIBDV infection caused a modulation of gut microbiota composition in the caecal content. This study clearly confirmed the pathogenesis of vvIBDV infection, and an immunosuppressive effect of vvIBDV on the CT and immune parameters in the caecum.

The aim of study 2 was to investigate the effects of vvIBDV on potential, secondary pathogens in the gut. We selected *C. jejuni* to follow up on this approach because it is considered as a commensal in healthy birds and may need cofactors to induce lesions and to lead to disease. Two experiments were conducted in study 2. The colonization pattern of *C. jejuni*, local immune responses in the BF and caecum as well as gut microbiota composition in the caecal content were compared between vvIBDV-and *C. jejuni*-mono-inoculated and vvIBDV+ *C. jejuni* co-inoculated birds. Depending on the time point of *C. jejuni* inoculation after vvIBDV-infection the colonization pattern of *C. jejuni* was modified differently. Bacterial inoculation at seven days pvi led to an increased number of CFU of *C. jejuni* during the early phase after inoculation, while bacterial inoculation at nine days pvi compromised the bacterial clearance. vvIBDV affected clearly the humoral immune response leading to a reduction of circulating B cells at the time of *C. jejuni* inoculation. Also later on local B cell

numbers in the BF and caecum were significantly reduced in comparison to virus-free controls ($P < 0.05$). The depression in B cells may have led to the significantly lower circulating anti-*C. jejuni* antibody levels, coinciding with a reduced clearance of the bacteria ($P < 0.05$).

8.1. vvIBDV-induced immunosuppression

Previous studies have demonstrated that the acute immunosuppressive phase induced by IBDV was accompanied by an infiltration of immune cells into the BF such as T lymphocytes and macrophages, a 'cytokine storm' as well as a depletion of B lymphocytes within the first week post challenge (Kim et al., 2000). In study 1, virus replication, severe lesion development including a depletion of B lymphocytes, accumulation of T lymphocytes and macrophages were observed in the BF, and also in the CT and caeum of vvIBDV inoculated birds. While the infiltration of heterophils and macrophage was only observed at three and seven dpi, an increase in the number of T lymphocytes and a depletion of B lymphocytes were observed in the BF, CT and caecum at least until 14 dpi (Rauw et al., 2007; Sharma et al., 2000; Withers et al., 2005). Comparable results were also obtained in study 2. The depletion of B cells was not only observed in the BF, but also, as demonstrated in study 1, in the CT and caecum. In study 2 additionally PBL were investigated confirming also a systemic B cell depletion from five to at least nine days pvi. In both studies, we observed virus clearance in the BF at around 21-23 days pvi coinciding with the beginning bursal recovery in the vvIBDV-inoculated birds. We observed that the antibody response against *C. jejuni* was suppressed between 14 and 21 days pbi (23 and 30 days pvi) in the co-inoculated birds compared to *C. jejuni*-mono-inoculated birds. Our results clearly show that IBDV-induced immunosuppression locally and systemically lasted beyond the peak of viral replication and even beyond 30 days pvi, when bursal recovery and antigen clearance were observed.

8.2. Innate and acquired gut associated immunity and defense

It was suggested that the mucosa may directly regulate adaptive immune responses and maintain the homeostasis between gut pathogens and host immunity (MacDonald and Monteleone, 2005; Neutra et al., 1996). The intestinal tract consists of immune tissues and is important as a first barrier for the host defense against invading pathogens (MacDonald and Monteleone, 2005). The function of the GALT mainly depends on the collaboration of

immune cells, such as T cells, B cells and mast cells (Koboziev et al., 2010). As innate immune cells, mast cells, play an important role in the local mucosal immune response during enteric infection (Caldwell et al., 2004; Metcalfe et al., 1997). We observed a decrease in the number of mast cells in the caecum of vvIBDV-inoculated birds compared to virus-free controls in both studies. We speculate that this decrease contributes to an impaired gut immunity (Wang et al., 2009b).

The target cells of IBDV are B cells. Field studies indicated that the common infection route with IBDV is via the oral route. Some immune cells in the intestine such as lymphocytes and macrophages are suggested to be involved in the viral transmission. IBDV antigen was observed in the jejunum and duodenum as early as four hpi and could be detected in the cloacal bursa as early as 11 hpi. In study 1, IBDV antigen was detected in the BF, CT as well as caecum. Virus replication mainly occurred in the BF and peaked between three and five dpi. Only a few virus-antigen-positive cells were observed in the CT and caecum of vvIBDV-inoculated birds. The germinal centers in the CT and caecum have a large number of B cells, which support the virus replication. IBDV infection was accompanied by histological lesions. We observed an infiltration of heterophils in the lamina propria in the CT and caecum, and a reduction of caecal total mucosal thickness. A significant reduction of caecal total mucosal thickness was observed starting at seven dpi. It can be speculated that the lesions in the CT and caecum could be due to the secondary viremia following excessive replication of IBDV in the BF (Olah and Glick, 1979).

During IBDV infection, T cells might play a role in virus clearance, but also contribute to tissue damage in the BF (Rautenschlein et al., 2002). In both studies, a significant increase in T cell numbers was observed in the BF of vvIBDVinfected birds compared to virus-free controls, coinciding with previous studies (Rautenschlein et al., 2007; Tanimura and Sharma, 1997). In the caecum, virusl-antigen-positive cells were mainly observed in the germinal center. The increase in the number of T LPL might due to the movement of CD4+ and CD8ß+ lymphocyte from the intraepithelial to submucosal areas, which may explain the decrease in the number of T IEL in vvIBDV-inoculated birds compared to virus-free control ($P < 0.05$). We speculate that the increase of T LPL in the caecum of vvIBDV-infected chickens contribute to the virus-clearance and correlate with the protective immunity in the gut (Kim et al., 1999).

8.3. Effect of vvIBDV and *C. jejuni* on gut microbiota

There is limited literature on dysbiosis caused by viruses. Recent studies have indicated that viruses such as human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and influenza virus could influence the gut microbiota composition (Handley et al., 2012; Lozupone et al., 2013). In study 1, our results demonstrated that vvIBDV-infection led to changes in gut microbiota composition and these changes varied over time. A lower abundance of *Clostridium XIVa* was observed at three dpi in the caecal content of vvIBDV-inoculated birds compared to virus-free birds. However, starting at seven dpi, a higher abundance of *Clostridium XIVa* was observed in vvIBDV inoculated birds in comparison to virus-free ones. In addition, we also observed that vvIBDV infection led to an increase in *Faecalibacterium* at seven dpi, followed by a decrease at 14 and 21 dpi. One recent study indicate that MDV-infection also modified the gut microbiota composition over time, and these changes might be associated with the lifecycle of virus (Perumbakkam et al., 2014). It is unknown whether there is an interaction between the lifecycle of IBDV and gut microbiota composition. The modulation of the gut microbiota composition might be associated with the functionality of the immune system. During IBDV-infection, the acute phase is characterized with a quick viral replication, a strong inflammatory response with a so called 'cytokine storm' between three and seven dpi. After the acute phase, all these reactions decrease over time.

Previous study demonstrated that *Clostridium.* spp are strong inducers of colonic T regulatory (Treg) cells (Atarashi et al., 2011). A higher abundance of *Clostridium* XIVa was observed at 3 dpi in the caecum of vvIBDV-inoculated birds compared to virus-free controls. We speculate that the higher abundance of *Clostridium* XIVa might be an indicator of an acute immunosuppression. A decrease in the abundance of *Clostridium XIVa* had been observed in inflammatory bowel disease (IBD) patients compared to healthy subjects (Frank et al., 2007; Sokol et al., 2008; Willing et al., 2009). In our study, the decrease in the abundance of *Clostridium* XIVa at 14 and 21 dpi in the vvIBDV-inoculated birds might suggest that vvIBDV interferes with the delicate balance of gut mucosal immunity.

The role of *Faecalibacterium* was unkown in chicken. In human it was demonstrated that *Faecalibacterium prausnitzii* is a sensor and a marker of human health (Sokol et al., 2008). A diminished abundance of *Faecalibacterium prausnitzii* is associated with IBD (Manichanh et

al., 2006), colorectal cancer (Sobhani et al., 2011). If this theory can be transferred to chickens study, we may speculate that vvIBDV also leads to intestinal disorders.

The role of the gut microbiota composition in *C. jejuni* infection was already shown in chickens. Germ-free as well as antibiotic-treated chickens were more sensitive to *C. jejuni* infection. These chickens developed a higher colonization rate and a stronger immune response of T cells and B cells in the caecum and BF compared to commercial chickens (Han et al., 2017). Our studies clearly indicate that vvIBDV-infection leads to a modulation of the gut microbiota composition, which may subsequently affect the colonization of *C. jejuni*. Our findings combined with previous studies further confirmed that the gut microbiota plays an important role in the pathogenesis of *C. jejuni*. *Faecalibacterium* is a butyrate producer (Duncan et al., 2004). Previous study suggested that high numbers of *Faecalibacterium* may be detrimental for *C. jejuni* since butyrate may inhibit replication of *C. jejuni* (Van Deun et al., 2008c). In study 2, different colonization rates between *C. jejuni* mono-inoculated and co-inoculated birds might be due to different abundance of *Faecalibacterium* in the gut microbiota. A lower abundance of *Faecalibacterium* was observed in vvIBDV-inoculated birds, which coincided with higher colonization rates of *C. jejuni* in the co-inoculated birds. Further studies on the interaction with certain gut microbiota components and *C. jejuni* need to be conducted to elucidate the exact mechanisms involved in the control of *C. jejuni* in the gut.

8.4. Effect of vvIBDV on *C. jejuni*

It is becoming increasingly controversial to define whether *C. jejuni* is a commensal or a pathogen in chickens. However, an increasing number of studies showed that the immune status of the host affects the pathogenesis of *C. jejuni*. Recently, two studies demonstrated that IBDV inoculation could exacerbate the colonization of *C. jejuni* in the ceacum. Subler et al. demonstrated that *C. jejuni*-shedding increased after infection of SPF chickens with an IBDV Del-E and subsequent inoculation of *C. jejuni* at 14 days post virus infection In another study, immunization with an attenuated IBDV live vaccine and immediate inoculation with *C. jejuni*, led to more severe lesions in the liver and intestine (Stojanov et al., 2008). Significantly lower *C. jejuni*-specific antibody titers were observed in co-inoculated chickens compared to *C. jejuni*-mono-inoculated birds (Stojanov et al., 2008). In study 2, two different

time intervals were selected between the inoculation of vvIBDV and *C. jejuni*. Our results showed that vvIBDV-induced immunosuppression clearly influenced the colonization of *C. jejuni*. Moreover, to some extent vvIBDV infection could delay the clearance of *C. jejuni*. A significant difference was observed in the number of *C. jejuni*-positive birds and CFU between co-inoculated and *C. jejuni*-mono-inoculated birds at 21 days pbi. We may speculate that this delay would even be more clearly visible if the experiment would have been extended. Interestingly, an inoculation interval with a difference of two days modified the colonization pattern of *C. jejuni*. The vvIBDV-induced cytokine storm may affect the pathogenesis of *C. jejuni* during the early phase (Exp. A), while advanced immunosuppression and compromised humoral immunity may more affect the clearance phase (Exp. B) (Sharma et al., 2000; Tippenhauer et al., 2013; Vervelde and Davison, 1997; Withers et al., 2006). It was suggested that circulating IgG and local IgA could play a role in controlling *C. jejuni* (Cawthraw et al., 2000; Myszewski and Stern, 1990; Perlman et al., 1988). In study 2, a significant lower level of circulating anti-*C. jejuni* IgG specific antibodies was observed at 21 dpi in the co-inoculated birds compared to *C. jejuni* mono-inoculated birds ($P < 0.05$). The suppressed IgG antibody level coincided with a significant higher number of CFU in the co-inoculated compared to *C. jejuni* mono-inoculated birds. This provides circumstandance evidence that humoral immunity could play a role in control of *C. jejuni* in birds.

8.5. Critical evaluation of the experimental approach in these studies and future perspectives

It is known that broiler chickens serve as a potential reservoir for *Campylobacter* strains, which are pathogenic to humans (Altekruse et al., 1999; Friis et al., 2010). They are considered as the main reservoir for food contamination. Therefore, broiler chickens were used in these two studies to be as close as possible to the field situation. Although broiler chickens were inoculated when MDA were below the breakthrough level of the virus, some remaining interfering effects of low MDA levels may have occurred during vvIBDV infection. In addition, birds were already 14 day old at the time of vvIBDV infection. To better evaluate the effect of IBDV on the early development of GALT as well as the gut microbiota, it may be necessary to select broiler chickens which are negative for MDA and infected them at or shortely after hatch.

vvIBDV infection usually leads to strong immune responses, including inflammatory responses (IFN-γ, IL-6. IL-8). These strong immune responses, especially during the acute phase of the disease might affect the pathogenesis of *C. jejuni*. Therefore, to evaluate the role of humoral immunity on *C. jejuni* for example in a humoral immunosuppression model such as IBD, inoculation of *C. jejuni* should be performed at later time points, possibly at 14 days post vvIBDV infection, when a reduction of anti-*C. jejuni* antibody levels can be clearly detected.

In the near future, we would like to investigate if probiotics are suitable to control the effects of IBDV on the GALT and microbiota or *C. jejuni* infection. Daily oral administration of *Lactobacillus (L.) plantarum* L-137, *L. fermentum* CECT5716 *or Bifidobacterium (B.) casei* DN114-001, *B. fermentum* CECT5716 before and after influenza virus H1N1 challenge in mice enhanced survival and decreased virus titers in lungs of infected mice (Maeda et al., 2009). Some probiotics such as *L. acidophilus* strain NCFM, *B. animalis* subsp. *lactis* BI-07 were shown to reduce influenza-like symptoms (Leyer et al., 2009). In previous studies some probiotics have been investigated in chickens, such as *L. acidophilus* and *Enterococcus* (*E.*) faecium, *L. acidophilus, L. casei, B. thermophilus*, *B. longum* PCB 133, which were shown to reduce the colonization of *C. jejuni* in chickens. This might provide a novel insight into the control of gut infections.

Since IBD-induced immunosuppression and gut-associated diseases are common in the field, the results of this study provide knowledge about the interaction between IBDV-infection and the development the gut-associated immune system. Understanding the mechanism of *C. jejuni* infection in poultry is crucial to eliminate the risk of C. *jejuni* to public health. Since broilers chickens are one of the main sources for a food-related *C. jejuni* infection in humans, we believe that stronger monitoring of immunosuppressive agents, such as IBDV in chickens, may be useful in improving control of *Campylobacter* infections in consumers.

9. References

Ahmed, I.H., Manning, G., Wassenaar, T.M., Cawthraw, S., Newell, D.G., 2002. Identification of genetic differences between two Campylobacter jejuni strains with different colonization potentials. Microbiol 148, 1203-1212.

Al-Natour, M., Ward, L., Saif, Y.M., Stewart-Brown, B., Keck, L., 2004. Effect of different levels of maternally derived antibodies on protection against infectious bursal disease virus. Avian Dis 48, 177-182.

Allan, W.H., Faragher, J.T., Cullen, G.A., 1972. Immunosuppression by the infectious bursal agent in chickens immunised against Newcastle disease. Vet Rec 90, 511-512.

Altekruse, S.F., Stern, N.J., Fields, P.I., Swerdlow, D.L., 1999. Campylobacter jejuni--an emerging foodborne pathogen. Emerg Infect Dis 5, 28.

Anderson, W.I., Reid, W.M., Lukert, P.D., Fletcher Jr, O.J., 1977. Influence of infectious bursal disease on the development of immunity to Eimeria tenella. Avian Dis, 637-641.

Apajalahti, J., Kettunen, A., Graham, H., 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. Worlds Poult Sci J 60, 223.

Aregitu, M., 2015. Outbreak investigation and molecular characterization of infectious bursal disease virus and vaccine immunogenesity trial in ethiopia.

Aricibasi, M., Jung, A., Heller, E.D., Rautenschlein, S., 2010. Differences in genetic background influence the induction of innate and acquired immune responses in chickens depending on the virulence of the infecting infectious bursal disease virus (IBDV) strain. Vet Immunol Immunopathol 135, 79-92.

Arsi, K., Donoghue, A., Woo-Ming, A., Blore, P., Donoghue, D., 2015. Intracloacal inoculation, an effective screening method for determining the efficacy of probiotic bacterial isolates against Campylobacter colonization in broiler chickens. J Food Prot 78, 209-213.

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., 2011. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331, 337-341.

Awad, W., Dublecz, F., Hess, C., Dublecz, K., Khayal, B., Aschenbach, J., Hess, M., 2016a. Campylobacter jejuni colonization promotes the translocation of Escherichia coli to extra-intestinal organs and disturbs the short-chain fatty acids profiles in the chicken gut. Poult Sci 95, 2259-2265.

Awad, W.A., Mann, E., Dzieciol, M., Hess, C., Schmitz-Esser, S., Wagner, M., Hess, M., 2016b. Age-related differences in the luminal and mucosa-associated gut microbiome of broiler chickens and shifts associated with Campylobacter jejuni infection. Front Cell Infect Microbiol 6.

Awad, W.A., Smorodchenko, A., Hess, C., Aschenbach, J.R., Molnár, A., Dublecz, K., Khayal, B., Pohl, E.E., Hess, M., 2015. Increased intracellular calcium level and impaired nutrient absorption are important pathogenicity traits in the chicken intestinal epithelium during Campylobacter jejuni colonization. Appl Microbiol Biotechnol 99, 6431-6441.

Azad, A.A., Barrett, S., Fahey, K., 1985. The characterization and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus. Virol 143, 35-44.

Azad, A.A., Jagadish, M.N., Brown, M.A., Hudson, P.J., 1987. Deletion mapping and expression in Escherichia coli of the large genomic segment of a birnavirus. Virol 161, 145-152.

Bar-Shira, E., Sklan, D., Friedman, A., 2003. Establishment of immune competence in the avian GALT during the immediate post-hatch period. Dev Comp Immunol 27, 147-157.

Barnes, E.M., Impey, C.S., Cooper, D.M., 1980. Manipulation of the crop and intestinal flora of the newly hatched chick. Am J Clin Nutr 33, 2426-2433.

Bautista, D., Elankumaran, S., Heckert, R., 2004. Effect of a variant infectious bursal disease virus (E/Del) on Salmonella typhimurium infection in commercial broiler chickens. Avian Dis 48, 361-369.

Beagley, K., Husband, A.J., 1998. Intraepithelial lymphocytes: origins, distribution, and function. Crit Rev Immunol 18, 237-254.

Becht, H., Müller, H., Müller, H.K., 1988. Comparative studies on structural and antigenic properties of two serotypes of infectious bursal disease virus. J Gen Virol 69 (Pt 3), 631-640.

Befus, A.D., Johnston, N., Leslie, G., Bienenstock, J., 1980. Gut-associated lymphoid tissue in the chicken. I. Morphology, ontogeny, and some functional characteristics of Peyer's patches. J Immunol 125, 2626-2632.

Benton, W., Cover, M., Rosenberger, J., 1967. Studies on the transmission of the infectious bursal agent (IBA) of chickens. Avian Dis 11, 430-438.

Berg, T.P., 2000. Acute infectious bursal disease in poultry: a review. Avian Pathol 29, 175-194.

Berkes, J., Viswanathan, V.K., Savkovic, S.D., Hecht, G., 2003. Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. Gut 52, 439-451.

Binek, M., Borzemska, W., Pisarski, R., Kosowska, G., Malec, H., Karpińska, E., 2000. Evaluation of the efficacy of feed providing on development of gastrointestinal microbiota of newly hatched broiler chickens. Archiv für Geflügelkunde 64, 147-151.

Birghan, C., Mundt, E., Gorbalenya, A.E., 2000. A non-canonical Lon proteinase lacking the ATPase domain employs the Ser–Lys catalytic dyad to exercise broad control over the life cycle of a double-stranded RNA virus. EMBO J 19, 114-123.

Blakey, J.L., Lubitz, L., Barnes, G., Bishop, R.F., Campbell, N., Gillam, G., 1982. Development of gut colonisation in pre-term neonates. J Med Microbiol 15, 519-529.

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

Boshuizen, J.A., Reimerink, J.H., Korteland-van Male, A.M., van Ham, V.J., Bouma, J., Gerwig, G.J., Koopmans, M.P., Büller, H.A., Dekker, J., Einerhand, A.W., 2005. Homeostasis and function of goblet cells during rotavirus infection in mice. Virol 337, 210-221.

Brandt, M., Yao, K., Liu, M., Heckert, R.A., Vakharia, V.N., 2001. Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. J Virol 75, 11974-11982.

Brenner, D.J., Farmer, J., 1984. Enterobacteriaceae. Bergey's Manual of Systematics of Archaea and Bacteria.

Busnadiego, I., Maestre, A.M., Rodríguez, D., Rodríguez, J.F., 2012. The infectious bursal disease virus RNA-binding VP3 polypeptide inhibits PKR-mediated apoptosis. PloS one 7, e46768.

Caldwell, D., Danforth, H., Morris, B., Ameiss, K., McElroy, A., 2004. Participation of the intestinal epithelium and mast cells in local mucosal immune responses in commercial poultry. Poult Sci 83, 591-599.

Calneck, B. 1997. infectious bursal disease: DiseasesofPoultry, 10" ed (P).

Cambra-López, M., Hermosilla, T., Lai, H., Aarnink, A., Ogink, N., 2011. Particulate matter emitted from poultry and pig houses: source identification and quantification. Trans ASABE 54, 629-642.

Campbell, G. 2001. Investigation into evidence of exposure to infectious bursal disease virus (IBDV) and chick infectious anaemia virus (CIAV) in wild birds in Ireland. In: Proc. 2nd Int. Symp. Infectious Bursal Disease and Chicken Infectious Anaemia, Rauischholzhausen, Germany. Institut für Geflügelkrankheiten, Justus Liebig University, Giessen, Germany, 230-233.

Carballeda, J.M., Maroniche, G., Lucero, M.S., Richetta, M., Gómez, E., Zoth, S.C., Berinstein, A., 2015. Infectious Bursal Disease Virus non-structural protein VP5 is not a transmembrane protein. Virol 483, 312-317.

Casteleyn, C., Doom, M., Lambrechts, E., Van Den Broeck, W., Simoens, P., Cornillie, P., 2010. Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. Avian Pathog 39, 143-150.

Castón, J.R., Martínez-Torrecuadrada, J.L., Maraver, A., Lombardo, E., Rodríguez, J.F., Casal, J.I., Carrascosa, J.L., 2001. C terminus of infectious bursal disease virus major capsid protein VP2 is involved in definition of the T number for capsid assembly. J Virol 75, 10815-10828.

Cawthraw, S., Lind, L., Kaijser, B., Newell, D., 2000. Antibodies, directed towards Campylobacter jejuni antigens, in sera from poultry abattoir workers. Clinical & Experimental Immunol 122, 55-60.

Cawthraw, S., Newell, D., 2010. Investigation of the presence and protective effects of maternal antibodies against Campylobacter jejuni in chickens. Avian Dis 54, 86-93.

Cerutti, A., Cols, M., Puga, I., 2013. Marginal zone B cells: virtues of innatelike antibody-producing lymphocytes. Nat Rev Immunol 13, 118.

Chaloner, G., Wigley, P., Humphrey, S., Kemmett, K., Lacharme-Lora, L., Humphrey, T., Williams, N., 2014a. Dynamics of dual infection with Campylobacter jejuni strains in chickens reveals distinct strain-to-strain variation in infection ecology. Appl Environ Microbiol 80, 6366-6372.

Chen, Y.-Y., Hsieh, M.K., Tung, C.-Y., Wu, C.C., Lin, T.L., 2011. Infectious bursal disease DNA vaccination conferring protection by delayed appearance and rapid clearance of invading viruses. Arch Virol 156, 2241-2250.

Chen, Y., Lu, Z., Zhang, L., Gao, L., Wang, N., Gao, X., Wang, Y., Li, K., Gao, Y., Cui, H., 2016. Ribosomal protein L4 interacts with viral protein VP3 and regulates the replication of infectious bursal disease virus. Virus Res 211, 73-78.

Chettle, N., Stuart, J., Wyeth, P., 1989. Outbreak of virulent infectious bursal disease in East Anglia. Vet Rec 125, 271-272.

Chevalier, C., Lepault, J., Da Costa, B., Delmas, B., 2004. The last C-terminal residue of VP3, glutamic acid 257, controls capsid assembly of infectious bursal disease virus. J Virol 78, 3296-3303.

Chevalier, C., Lepault, J., Erk, I., Da Costa, B., Delmas, B., 2002. The maturation process of pVP2 requires assembly of infectious bursal disease virus capsids. J Virol 76, 2384-2392.

Cheville, N.F., 1967. Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen, and thymus of the chicken. Am J Pathol 51, 527.

Cho, B., 1970. Experimental dual infections of chickens with infectious bursal and Marek's disease agents I. Preliminary observation on the effect of infectious bursal agent on Marek's disease. Avian Dis, 665-675.

Choct, M., Hughes, R., Wang, J., Bedford, M., Morgan, A., Annison, G., 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br Poult Sci 37, 609-621.

Cloud, S., Lillehoj, H., Rosenberger, J., 1992a. Immune dysfunction following infection with chicken anemia agent and infectious bursal disease virus. I. Kinetic alterations of avian lymphocyte subpopulations. Vet Immunol Immunopathol 34, 337-352.

Cloud, S., Rosenberger, J., Lillehoj, H., 1992b. Immune dysfunction following infection with chicken anemia agent and infectious bursal disease virus. II. Alterations of in vitro lymphoproliferation and in vivo immune responses. Vet Immunol Immunopathol 34, 353-366.

Cole, K., Farnell, M., Donoghue, A., Stern, N., Svetoch, E., Eruslanov, B., Volodina, L., Kovalev, Y., Perelygin, V., Mitsevich, E., 2006. Bacteriocins reduce Campylobacter colonization and alter gut morphology in turkey poults. Poult Sci 85, 1570-1575.

Confer, A., MacWilliams, P., 1982. Correlation of hematological changes and serum and monocyte inhibition with the early suppression of phytohemagglutinin stimulation of lymphocytes in experimental infectious bursal disease. Can J Comp Med 46, 169.

Cosgrove, A., 1962. An apparently new disease of chickens: avian nephrosis. Avian Dis 6, 385-389.

Cox, H., Moffatt, M., Faux, J., Walley, A., Coleman, R., Trembath, R., Cookson, W., Harper, J., 1998. Association of atopic dermatitis to the beta subunit of the high affinity immunoglobulin E receptor. Br J Dermatol 138, 182-187.

Crhanova, M., Hradecka, H., Faldynova, M., Matulova, M., Havlickova, H., Sisak, F., Rychlik, I., 2011. Immune response of chicken gut to natural colonization by gut microbiota and to Salmonella enterica serovar enteritidis infection. Infect Immun 79, 2755-2763.

Danzeisen, J.L., Kim, H.B., Isaacson, R.E., Tu, Z.J., Johnson, T.J., 2011. Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. PloS one 6, e27949.

Dasso, J.F., Obiakor, H., Bach, H., Anderson, A.O., Mage, R.G., 2000. A morphological and immunohistological study of the human and rabbit appendix for comparison with the avian bursa. Dev Comp Immunol 24, 797-814.

Dasti, J.I., Tareen, A.M., Lugert, R., Zautner, A.E., Gross, U., 2010. Campylobacter jejuni: a brief overview on pathogenicity-associated factors and disease-mediating mechanisms. Int J Med Microbiol 300, 205-211.

De Wit, J. 2001. Gumboro disease: estimation of optimal time of vaccination by the Deventer formula. In: Proceedings of the 3rd meeting of working group, 21-28.

Degnan, B.A., Macfarlane, G.T., 1991. Comparison of carbohydrate substrate preferences in eight species of bifidobacteria. FEMS Microbiol letters 84, 151-156.

Del Moral, M.G., Fonfria, J., Varas, A., Jimenez, E., Moreno, J., Zapata, A., 1998. Appearance and development of lymphoid cells in the chicken(Gallus gallus) caecal tonsil. Anat Rec 250, 182-189.

Deplancke, B., Gaskins, H.R., 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73, 1131S-1141S.

Dhillon, A.S., Shivaprasad, H., Schaberg, D., Wier, F., Weber, S., Bandli, D., 2006. Campylobacter jejuni infection in broiler chickens. Avian Dis 50, 55-58.

Dillon, S., Lee, E., Kotter, C., Austin, G., Dong, Z., Hecht, D., Gianella, S., Siewe, B., Smith, D., Landay, A., 2014. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol 7, 983-994.

Dimitriu, P.A., Boyce, G., Samarakoon, A., Hartmann, M., Johnson, P., Mohn, W.W., 2013. Temporal stability of the mouse gut microbiota in relation to innate and adaptive immunity. Environ Microbiol Rep 5, 200-210.

Dobos, P., 1976. Size and structure of the genome of infectious pancreatic necrosis virus. Nucleic Acids Res 3, 1903-1924.

Dobos, P., 1979. Peptide map comparison of the proteins of infectious bursal disease virus. J Virol 32, 1047-1050.

Dohms, J., Lee, K., Rosenberger, J., 1981. Plasma cell changes in the gland of Harder following infectious bursal disease virus infection of the chicken. Avian Dis, 683-695.

Duncan, S.H., Hold, G.L., Harmsen, H.J., Stewart, C.S., Flint, H.J., 2002. Growth requirements and fermentation products of Fusobacterium prausnitzii, and a proposal to reclassify it as Faecalibacterium prausnitzii gen. nov., comb. nov. Int J Syst Evol Microbiol 52, 2141-2146.

Duncan, S.H., Holtrop, G., Lobley, G.E., Calder, A.G., Stewart, C.S., Flint, H.J., 2004. Contribution of acetate to butyrate formation by human faecal bacteria. Br J Nurtr 91, 915-923.

Dunon, D., Courtois, D., Vainio, O., Six, A., Chen, C., Cooper, M., Dangy, J., Imhof, B., 1997. Ontogeny of the immune system: γ/δ and α/β T cells migrate from thymus to the periphery in alternating waves. J Exp Med 186, 977-988.

Edwards, L.A., Nistala, K., Mills, D.C., Stephenson, H.N., Zilbauer, M., Wren, B.W., Dorrell, N., Lindley, K.J., Wedderburn, L.R., Bajaj-Elliott, M., 2010. Delineation of the innate and adaptive T-cell immune outcome in the human host in response to Campylobacter jejuni infection. PLoS One 5, e15398.

EFSA, A., 2013. Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2013. Scientific Opinion on the re-evaluation of aspartame (E 951) as a food additive. EFSA Journal 11, 3496.

EFSA, C., 2011. Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on Flavouring Group Evaluation 213.

Elankumaran, S., Heckert, R., Moura, L., 2002. Pathogenesis and tissue distribution of a variant strain of infectious bursal disease virus in commercial broiler chickens. Avian Dis 46, 169-176.

Eldaghayes, I., Rothwell, L., Williams, A., Withers, D., Balu, S., Davison, F., Kaiser, P., 2006. Infectious bursal disease virus: strains that differ in virulence differentially modulate the innate immune response to infection in the chicken bursa. Viral Immunol 19, 83-91.

Engberg, R.M., Hedemann, M.S., Leser, T., Jensen, B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microbiota and performance of broilers. Poult Sci 79, 1311-1319.

Engberg, R.M., Hedemann, M.S., Steenfeldt, S., Jensen, B.B., 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult Sci 83, 925-938.

Eterradossi, N., Saif, Y.M. 2008. Infectious bursal disease, In: Saif., Y.M., Fadly, A.M., Glission, J.R., McDougald, L.R., Nolan, L.K., Swayne, D.E. (Eds.) Diseases of Poultry12. Blackwell Publishing, Iowa, USA, 185-208.

Eterradossi, N., Toquin, D., Rivallan, G., Guittet, M., 1997. Modified activity of a VP2-located neutralizing epitope on various vaccine, pathogenic and hypervirulent strains of infectious bursal disease virus. Arch Virol 142, 255-270.

Fadly, A.M., Nazerian, K., 1983. Pathogenesis of infectious bursal disease in chickens infected with virus at various ages. Avian Dis 27, 714-723.

Fagerland, J., Arp, L., 1992. Distribution and quantitation of plasma cells, T lymphocyte subsets, and B lymphocytes in bronchus-associated lymphoid tissue of chickens: age-related differences. Reg Immunol 5, 28-36.

Fahey, K.J., Erny, K., Crooks, J., 1989. A conformational immunogen on VP-2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. J Gen Virol 70, 1473-1481.

Faragher, J.T., Allan, W.H., Wyeth, P.J., 1974. Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease. Vet Rec 95, 385-388.

Fernandez-Arias, A., Martinez, S., Rodriguez, J.F., 1997. The major antigenic protein of infectious bursal disease virus, VP2, is an apoptotic inducer. J Virol 71, 8014-8018.

Fimlaid, K.A., Lindow, J.C., Tribble, D.R., Bunn, J.Y., Maue, A.C., Kirkpatrick, B.D., 2014. Peripheral CD4+ T cell cytokine responses following human challenge and re-challenge with Campylobacter jejuni. PloS one 9, e112513.

Firth, G., 1974. Occurrence of an infectious bursal syndrome within an Australian poultry flock. Aust Vet J 50, 128-130.

Frank, D.N., Amand, A.L.S., Feldman, R.A., Boedeker, E.C., Harpaz, N., Pace, N.R., 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel disease. Proc Natl Acad Sci 104, 13780-13785.

Friedman, A., Bar-Shira, E., Sklan, D., 2003. Ontogeny of gut associated immune competence in the chick. Worlds Poult Sci J 59, 209-219.

Friis, C., Wassenaar, T.M., Javed, M.A., Snipen, L., Lagesen, K., Hallin, P.F., Newell, D.G., Toszeghy, M., Ridley, A., Manning, G., 2010. Genomic characterization of Campylobacter jejuni strain M1. PloS one 5, e12253.

Fuller, R., 1973. Differences in the microbiotas of the intestine and the bursa of fabricius as illustrated by the coliform/lactobacillus ratio in the two sites. Br Poult Sci 14, 221–224

Gao, H., Li, K., Gao, L., Qi, X., Gao, Y., Qin, L., Wang, Y., Wang, X., 2013. DNA prime–protein boost vaccination enhances protective immunity against infectious bursal disease virus in chickens. Vet Microbiol 164, 9-17.

Gardner, H., Knowles, K., Riddle, M., Brouwer, S., Gleeson, L., 1997. Poultry virus infection in Antarctic penguins. Nature 387:245

Gasc, J.-M., Stumpf, W.E., 1981. The bursa of Fabricius of the chicken embryo: localization and ontogenic evolution of sex-steroid target cells. Development 63, 225-231.

Geyra, A., Uni, Z., Sklan, D., 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult Sci 80, 776-782.

Ghareeb, K., Awad, W., Mohnl, M., Schatzmayr, G., Boehm, J., 2013. Control strategies for Campylobacter infection in poultry production. Worlds Poult Sci J 69, 57-76.

Gong, J., Forster, R.J., Yu, H., Chambers, J.R., Sabour, P.M., Wheatcroft, R., Chen, S., 2002a. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. FEMS Microbiol letters 208, 1-7.

Gong, J., Forster, R.J., Yu, H., Chambers, J.R., Wheatcroft, R., Sabour, P.M., Chen, S., 2002b. Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum. FEMS Microbiol Ecol 41, 171-179.

Gong, J., Si, W., Forster, R.J., Huang, R., Yu, H., Yin, Y., Yang, C., Han, Y., 2007. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. FEMS Microbiol Ecol 59, 147-157.

Gong, J., Yu, H., Liu, T., Gill, J., Chambers, J., Wheatcroft, R., Sabour, P., 2008. Effects of zinc bacitracin, bird age and access to range on bacterial microbiota in the ileum and caeca of broiler chickens. J Appl Microbiol 104, 1372-1382.

Gormley, F.J., MacRae, M., Forbes, K.J., Ogden, I.D., Dallas, J.F., Strachan, N.J., 2008. Has retail chicken played a role in the decline of human campylobacteriosis? Appl Environ Microbiol 74, 383-390.

Greenfield, C., Dohms, J., Dietert, R., 1986. Infectious bursal disease virus infection in the quail-chicken hybrid. Avian Dis, 536-542.

Grilli, E., Vitari, F., Domeneghini, C., Palmonari, A., Tosi, G., Fantinati, P., Massi, P., Piva, A., 2013. Development of a feed additive to reduce caecal Campylobacter jejuni in broilers at slaughter age: from in vitro to in vivo, a proof of concept. J Appl Microbiol 114, 308-317.

Guarner, F., Malagelada, J.-R., 2003. Gut flora in health and disease. The Lancet 361, 512-519.

Guo, X., Wang, L., Cui, D., Ruan, W., Liu, F., Li, H., 2012. Differential expression of the Toll-like receptor pathway and related genes of chicken bursa after experimental infection with infectious bursa disease virus. Arch Virol 157, 2189-2199.

Haas, B., Hinz, K.H., Glunder, G., 1999. Biotin-streptavidin enzyme-linked immunosorbent assay for the detection of antibodies to Campylobacter jejuni and C. coli in chickens. Zentralblatt fur Veterinarmedizin. Reihe B. J Vet Med. Series B 46, 163-171.

Hafez, H., Prusas, C., Raue, R., 2003. Very virulent infectious bursal disease virus (vvIBDV) in vaccinated broiler flock: Course of the disease, identification and characterisation of isolated strain. Archiv fur Geflugelkunde 67, 2-5.

Han, Z., Pielsticker, C., Gerzova, L., Rychlik, I., Rautenschlein, S., 2016a. The influence of age on Campylobacter jejuni infection in chicken. Dev Comp Immunol 62, 58-71.

Han, Z., Willer, T., Li, L., Pielsticker, C., Rychlik, I., Velge, P., Kaspers, B., Rautenschlein, S., 2017. The influence of the gut microbiota composition on Campylobacter jejuni colonization in chickens. Infect Immun, IAI. 00380-00317.

Han, Z., Willer, T., Pielsticker, C., Gerzova, L., Rychlik, I., Rautenschlein, S., 2016b. Differences in host breed and diet influence colonization by Campylobacter jejuni and induction of local immune responses in chicken. Gut Pathog 8, 56.

Handley, S.A., Thackray, L.B., Zhao, G., Presti, R., Miller, A.D., Droit, L., Abbink, P., Maxfield, L.F., Kambal, A., Duan, E., 2012. Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. Cell 151, 253-266.

Hänel, I., Borrmann, E., Müller, J., Müller, W., Pauly, B., Liebler-Tenorio, E., Schulze, F., 2009. Genomic and phenotypic changes of Campylobacter jejuni strains after passage of the chicken gut. Vet Microbiol 136, 121-129.

Hassan, M.K., Al-Natour, M., Ward, L., Saif, Y., 1996. Pathogenicity, attenuation, and immunogenicity of infectious bursal disease virus. Avian Dis, 567-571.

Helmoldt, C., Garner, E., 1964. Experimentally induced Gumboro disease. Avain Dis 8, 561-575.

Henzler, D.J., Kradel, D.C., Davison, S., Ziegler, A.F., Singletary, D., DeBok, P., Castro, A.E., Lu, H., Eckroade, R., Swayne, D., Lagoda, W., Schmucker, B., Nesselrodt, A., 2003. Epidemiology, production losses, and control measures associated with an outbreak of avian influenza subtype H7N2 in Pennsylvania (1996-98). Avian Dis 47, 1022-1036.

Hepworth, P.J., Ashelford, K.E., Hinds, J., Gould, K.A., Witney, A.A., Williams, N.J., Leatherbarrow, H., French, N.P., Birtles, R.J., Mendonca, C., 2011. Genomic variations define divergence of water/wildlife-associated Campylobacter jejuni niche specialists from common clonal complexes. Environmental Microbiol 13, 1549-1560.

Hiraga, M., Nunoya, T., Otaki, Y., Tajima, M., Saito, T., Nakamura, T., 1994. Pathogenesis of highly virulent infectious bursal disease virus infection in intact and bursectomized chickens. J Vet Med Sci 56, 1057-1063.

Hirai, K., Funakoshi, T., Nakai, T., Shimakura, S., 1981. Sequential changes in the number of surface immunoglobulin-bearing B lymphocytes in infectious bursal disease virus-infected chickens. Avian Dis, 484-496.

Hirai, K., Shimakura, S., Kawamoto, E., Taguchi, F., Kim, S.T., Chang, C.N., Iritani, Y., 1974. The immunodepressive effect of infectious bursal disease virus in chickens. Avian Dis 18, 50-57.

Hoerr, F.J., 2010. Clinical aspects of immunosuppression in poultry. Avian Dis 54, 2-15.

Hooper, L.V., Littman, D.R., Macpherson, A.J., 2012. Interactions between the microbiota and the immune system. Science 336, 1268-1273.

Hovorková, P., Skřivanová, E., 2015. Use of Caprylic Acid in Broiler Chickens: Effect on Campylobacter jejuni. Foodborne pathogens and disease 12, 712-718.

Hovorková, P., Skřivanová, E., Kudrnová, E., Marounek, M., 2015. Effect Of Dietary Medium-Chain Fatty Acids On Campylobacter Jejuni In Broiler Chickens. Sci Agric Bohem 46, 154-158.

Hsieh, M.K., Wu, C.C., Lin, T.L., 2010. DNA-mediated vaccination conferring protection against infectious bursal disease in broiler chickens in the presence of maternal antibody. Vaccine 28, 3936-3943.

Hudson, P.J., McKern, N.M., Power, B.E., Azad, A.A., 1986. Genomic structure of tbe large RNA segment of infectious bursal disease virus. Nucleic Acids Res 14, 5001-5012.

Hulse, D.J., Romero, C.H., 2004. Partial protection against infectious bursal disease virus through DNA-mediated vaccination with the VP2 capsid protein and chicken IL-2 genes. Vaccine 22, 1249-1259.

Humphrey, S., Chaloner, G., Kemmett, K., Davidson, N., Williams, N., Kipar, A., Humphrey, T., Wigley, P., 2014. Campylobacter jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. mBio 5, e01364-01314.

Hung, C.-M., Yeh, C.-C., Chen, H.-L., Lai, C.-W., Kuo, M.-F., Yeh, M.-H., Lin, W., Tu, M.-Y., Cheng, H.-C., Chen, C.-M., 2010. Porcine lactoferrin administration enhances peripheral lymphocyte proliferation and assists infectious bursal disease vaccination in native chickens. Vaccine 28, 2895-2902.

Huo, S., Zuo, Y., Li, N., Li, X., Zhang, Y., Wang, L., Liu, H., Zhang, J., Cui, D., He, P., 2016. Chicken IL-7 as a potent adjuvant enhances IBDV VP2 DNA vaccine immunogenicity and protective efficacy. Vet Microbiol 193, 145-155.

Icard, A.H., Sellers, H.S., Mundt, E., 2008. Detection of infectious bursal disease virus isolates with unknown antigenic properties by reverse genetics. Avian Dis 52, 590-598.

Imai, K., Mase, M., Tsukamoto, K., Hihara, H., Yuasa, N., 1999. Persistent infection with chicken anaemia virus and some effects of highly virulent infectious bursal disease virus infection on its persistency. Res Vet Sci 67, 233-238.

Ingrao, F., Rauw, F., Lambrecht, B., van den Berg, T., 2013. Infectious Bursal disease: a complex host–pathogen interaction. Dev Comp Immunol 41, 429-438.

Ishikawa, N., Wakelin, D., Mahida, Y.R., 1997. Role of T helper 2 cells in intestinal goblet cell hyperplasia in mice infected with Trichinella spiralis. Gastroenterology 113, 542-549.

Islam, M., Das, B., Hossain, K., Lucky, N., Mostafa, M., 2003. A study on the occurrence of poultry diseases in Sylhet region of Bangladesh. Int J Poult Sci. 2, 354-356.

Islam, M.R., Zierenberg, K., Eterradossi, N., Toquin, D., Rivallan, G., Müller, H., 2001. Molecular and antigenic characterization of Bangladeshi isolates of infectious bursal disease virus demonstrate their similarities with recent European, Asian and African very virulent strains. J Vet Med B Infect Dis Vet Public Health 48, 211-221.

Ismail, N., Saif, Y., 1991. Immunogenicity of infectious bursal disease viruses in chickens. Avian Dis, 460-469.

Ismail, N., Saif, Y., Wigle, W., Havenstein, G., Jackson, C., 1990. Infectious bursal disease virus variant from commercial leghorn pullets. Avian Dis, 141-145.

Iván, J., Velhner, M., Ursu, K., Germán, P., Mató, T., Drén, C.N., Mészáros, J., 2005. Delayed vaccine virus replication in chickens vaccinated subcutaneously with an immune complex infectious bursal disease vaccine: quantification of vaccine virus by real-time polymerase chain reaction. Can J Vet Res 69, 135-142.

Ivanyi, J., Morris, R., 1976. Immunodeficiency in the chicken. IV. An immunological study of infectious bursal disease. Clinical and experimental Immunol 23, 154-165.

Jackwood, D.J., 2011. Viral competition and maternal immunity influence the clinical disease caused by very virulent infectious bursal disease virus. Avian Dis 55, 398-406.

Jeon, W.-J., Lee, E.-K., Joh, S.-J., Kwon, J.-h., Yang, C.-B., Yoon, Y.-S., Choi, K.-S., 2008. Very virulent infectious bursal disease virus isolated from wild birds in Korea: Epidemiological implications. Virus Res 137, 153-156.

Jeurissen, S., Vervelde, L., Janse, M., 1993. Structure and function of lymphoid tissues of the chicken. Poult Sci reviews 5, 183-207.

Jiang, J.Q., He, X.-S., Feng, N., Greenberg, H.B., 2008. Qualitative and quantitative characteristics of rotavirus-specific CD8 T cells vary depending on the route of infection. J Virol 82, 6812-6819.

Johnston, P., Liu, H., O'Connell, T., Phelps, P., Bland, M., Tyczkowski, J., Kemper, A., Harding, T., Avakian, A., Haddad, E., 1997. Applications in in ovo technology. Poult Sci 76, 165-178.

Jones, M.A., Marston, K.L., Woodall, C.A., Maskell, D.J., Linton, D., Karlyshev, A.V., Dorrell, N., Wren, B.W., Barrow, P.A., 2004. Adaptation of Campylobacter jejuni NCTC11168 to high-level colonization of the avian gastrointestinal tract. Infect Immun 72, 3769-3776.

Jozefiak, D., Rutkowski, A., Kaczmarek, S., Jensen, B.B., Engberg, R.M., Højberg, O., 2010. Effect of β-glucanase and xylanase supplementation of barley-and rye-based diets on caecal microbiota of broiler chickens. Br Poult Sci 51, 546-557.

Józefiak, D., Swiatkiewicz, S., Kieronczyk, B., Rawski, M., Dlugosz, J., Engberg, R.M., Hojberg, O., 2016. Clostridium perfringens challenge and dietary fat type modifies performance, microbiota composition and histomorphology of the broiler chicken gastrointestinal tract. Archiv Fuer Gefluegelkunde.

Jung, A., 2006. Pathogenesestudie eines intermediärvirulenten Gumborovirus in spezifiziert-pathogen-freien (SPF) Hühnern und kommerziellen Broiler. Diss., Klinik für Geflügel, Tierärztliche Hochschule, Hannover.

Jungmann, A., Nieper, H., Müller, H., 2001. Apoptosis is induced by infectious bursal disease virus replication in productively infected cells as well as in antigen-negative cells in their vicinity. J Gen Virol 82, 1107-1115.

Juricova, H., Videnska, P., Lukac, M., Faldynova, M., Babak, V., Havlickova, H., Sisak, F., Rychlik, I., 2013. Influence of Salmonella enterica serovar enteritidis infection on the development of the cecum microbiota in newly hatched chicks. Appl Environ Microbiol 79, 745-747.

Kaakoush, N.O., Sodhi, N., Chenu, J.W., Cox, J.M., Riordan, S.M., Mitchell, H.M., 2014. The interplay between Campylobacter and Helicobacter species and other gastrointestinal microbiota of commercial broiler chickens. Gut Pathog 6, 18.

Kasahara, Y., Chen, C.-L., Göbel, T., Pat Bucy, R., 1994. Intraepithelial lymphocytes in birds. advances in host defense mechanisms 9, 163-163.

Kaufer, I., Weiss, E., 1980. Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens. Infect Immun 27, 364-367.

Kawai, T., Akira, S., 2006. TLR signaling. Cell Death & Differentiation 13, 816-825.

Khan, W., 2008. Physiological changes in the gastrointestinal tract and host protective immunity: learning from the mouse-Trichinella spiralis model. Parasitology 135, 671-682.

Khatri, M., Palmquist, J.M., Cha, R.M., Sharma, J.M., 2005. Infection and activation of bursal macrophages by virulent infectious bursal disease virus. Virus Res 113, 44-50.

Khatri, M., Sharma, J.M., 2006. Infectious bursal disease virus infection induces macrophage activation via p38 MAPK and NF-kappaB pathways. Virus Res 118, 70-77.

Kibenge, F.S., Dhama, V., 1997. Evidence that virion-associated VP1 of avibirnaviruses contains viral RNA sequences. Arch Virol 142, 1227-1236.

Kibenge, F.S., Dhillon, A.S., Russell, R.G., 1988. Identification of serotype II infectious bursal disease viral proteins. Avian Pathol 17, 679-687.

Kibenge, F.S., Jackwood, D.J., Mercado, C.C., 1990. Nucleotide sequence analysis of genome segment A of infectious bursal disease virus. J Gen Virol 71 (Pt 3), 569-577.

Kim, I.-J., Gagic, M., Sharma, J.M., 1999. Recovery of antibody-producing ability and lymphocyte repopulation of bursal follicles in chickens exposed to infectious bursal disease virus. Avian Dis, 401-413.

Kim, I.-J., Karaca, K., Pertile, T., Erickson, S., Sharma, J., 1998. Enhanced expression of cytokine genes in spleen macrophages during acute infection with infectious bursal disease virus in chickens. Vet Immunol Immunopathol 61, 331-341.

Kim, I.-J., You, S.K., Kim, H., Yeh, H.-Y., Sharma, J.M., 2000. Characteristics of bursal T lymphocytes induced by infectious bursal disease virus. J Virol 74, 8884-8892.

Kitagawa, H., Hiratsuka, Y., Imagawa, T., Uehara, M., 1998. Distribution of lymphoid tissue in the caecal mucosa of chickens. J Anat 192, 293-298.

Kitagawa, H., Shiraishi, S., Imagawa, T., Uehara, M., 2000. Ultrastructural characteristics and lectin-binding properties of M cells in the follicle-associated epithelium of chicken caecal tonsils. J Anat 197, 607.

Kizerwetter-Świda, M., Binek, M., 2008. Bacterial microbiota of the chicken embryos and newly hatched chicken. J Anim Feed Sci 17, 224-232.

Knarreborg, A., Simon, M.A., Engberg, R.M., Jensen, B.B., Tannock, G.W., 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl Environ Microbiol 68, 5918-5924.

Knudsen, K.N., Bang, D.D., Andresen, L.O., Madsen, M., 2006. Campylobacter jejuni strains of human and chicken origin are invasive in chickens after oral challenge. Avian Dis 50, 10-14.

Koboziev, I., Karlsson, F., Grisham, M.B., 2010. Gut-associated lymphoid tissue, T cell trafficking, and chronic intestinal inflammation. Ann N Y Acad Sci 1207.

Kosiewicz, M.M., Dryden, G.W., Chhabra, A., Alard, P., 2014. Relationship between gut microbiota and development of T cell associated disease. FEBS Lett 588, 4195-4206.

Kumar, P.A., Das, S.K., Rao, J.R., 1998. Effect of immunostimulation on cytotoxic activity of intestinal intraepithelial lymphocytes of chickens in infectious bursal disease and Eimeria tenella infections. Acta Vet Hung 46, 1-11.

Kumar, R., Charan, S., 2001. Virus enhancement following infection with antibody-coated infectious bursal disease virus (IBDV) in chickens. Indian J Exp Biol 39, 1314-1317.

Kumar, S., Ahi, Y.S., Salunkhe, S.S., Koul, M., Tiwari, A.K., Gupta, P.K., Rai, A., 2009. Effective protection by high efficiency bicistronic DNA vaccine against infectious bursal disease virus expressing VP2 protein and chicken IL-2. Vaccine 27, 864-869.

Lacharme-Lora, L., Chaloner, G., Gilroy, R., Humphrey, S., Gibbs, K., Jopson, S., Wright, E., Reid, W., Ketley, J., Humphrey, T., 2017. B lymphocytes play a limited role in clearance of Campylobacter jejuni from the chicken intestinal tract. Sci Rep 7, 45090.

Lan, Y., Verstegen, M., Tamminga, S., Williams, B., 2005. The role of the commensal gut microbial community in broiler chickens. Worlds Poult Sci J 61, 95-104.

Lasher, H.N., Davis, V.S., 1997. History of Infectious Bursal Disease in the USA: The First Two Decades. Avian Dis, 11-19.

Lee, C.C., Wu, C.C., Lin, T.L., 2015. Role of chicken melanoma differentiation-associated gene 5 in induction and activation of innate and adaptive immune responses to infectious bursal disease virus in cultured macrophages. Arch Virol 160, 3021-3035.

Lee, M.D., Newell, D.G., 2006. Campylobacter in poultry: filling an ecological niche. Avian Dis 50, 1-9.

Lejal, N., Da Costa, B., Huet, J.-C., Delmas, B., 2000. Role of Ser-652 and Lys-692 in the protease activity of infectious bursal disease virus VP4 and identification of its substrate cleavage sites. J Gen Virol 81, 983-992.

Leong, J., Brown, D., Dobos, P., Kibenge, F., Ludert, J., Müller, H., Mundt, E., Nicholson, B., 2000. Family birnaviridae. Virus taxonomy: classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses, 481-490.

Ley, D.H., Yamamoto, R., Bickford, A.A., 1983. The pathogenesis of infectious bursal disease: serologic, histopathologic, and clinical chemical observations. Avian Dis 27, 1060-1085.

Leyer, G.J., Li, S., Mubasher, M.E., Reifer, C., Ouwehand, A.C., 2009. Probiotic effects on cold and influenza-like symptom incidence and duration in children. Pediatrics 124, e172-e179.

Li, J., Huang, Y., Liang, X., Lu, M., Li, L., Yu, L., Deng, R., 2003. Plasmid DNA encoding antigens of infectious bursal disease viruses induce protective immune responses in chickens: factors influencing efficacy. Virus Res 98, 63-74.

Li, X., Swaggerty, C., Kogut, M., Chiang, H., Wang, Y., Genovese, K., He, H., Pevzner, I., Zhou, H., 2011. Caecal transcriptome analysis of colonized and non-colonized chickens within two genetic lines that differ in caecal colonization by Campylobacter jejuni. Anim Genet 42, 491-500.

Li, X., Swaggerty, C.L., Kogut, M.H., Chiang, H.I., Wang, Y., Genovese, K.J., He, H., Zhou, H., 2010. Gene expression profiling of the local cecal response of genetic chicken lines that differ in their susceptibility to Campylobacter jejuni colonization. PLoS One 5, e11827.

Li, Z., Wang, Y., Xue, Y., Li, X., Cao, H., Zheng, S.J., 2012. Critical role for voltage-dependent anion channel 2 in infectious bursal disease virus-induced apoptosis in host cells via interaction with VP5. J Virol 86, 1328-1338.

Liebler-Tenorio, E.M., Pabst, R., 2006. MALT structure and function in farm animals. Vet Res 37, 257-280.

Lillehoj, H.S., Chung, K.S., 1992. Postnatal development of T-lymphocyte subpopulations in the intestinal intraepithelium and lamina propria in chickens. Vet Immunol Immunopathol 31, 347-360.

Lillehoj, H.S., Trout, J.M., 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to Eimeria parasites. Clinical Microbiol reviews 9, 349-360.

Lin, T.-W., Lo, C.-W., Lai, S.-Y., Fan, R.-J., Lo, C.-J., Chou, Y.-m., Thiruvengadam, R., Wang, A.H.-J., Wang, M.-Y., 2007. Chicken heat shock protein 90 is a component of the putative cellular receptor complex of infectious bursal disease virus. J Virol 81, 8730-8741.

Linna, T., Liden, S., 1969. Cell migration from the bone marrow to the spleen in young guinea pigs. Int Arch Allergy Immunol 35, 35-46.

Liu, M., Vakharia, V.N., 2004. VP1 protein of infectious bursal disease virus modulates the virulence in vivo. Virol 330, 62-73.

Liu, M., Vakharia, V.N., 2006. Nonstructural protein of infectious bursal disease virus inhibits apoptosis at the early stage of virus infection. J Virol 80, 3369-3377.

Liu, Y., Wei, Y., Wu, X., Yu, L., 2005. Preparation of ChIL-2 and IBDV VP2 fusion protein by baculovirus expression system. Cell Mol Immunol 2, 231-235.

Lombardo, E., Maraver, A., Castón, J.R., Rivera, J., Fernández-Arias, A., Serrano, A., Carrascosa, J.L., Rodriguez, J.F., 1999. VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. J Virol 73, 6973-6983.

Lombardo, E., Maraver, A., Espinosa, I., Fernández-Arias, A., Rodriguez, J.F., 2000. VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. Virol 277, 345-357.

Lozupone, C.A., Li, M., Campbell, T.B., Flores, S.C., Linderman, D., Gebert, M.J., Knight, R., Fontenot, A.P., Palmer, B.E., 2013. Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe 14, 329-339.

Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D., 2003a. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl Environ Microbiol 69, 6816-6824.

Lu, J., Plataniotis, K.N., Venetsanopoulos, A.N., 2003b. Regularized discriminant analysis for the small sample size problem in face recognition. Pattern Recognit Lett 24, 3079-3087.

Lucio, B., Hitchner, S., 1980. Immunosuppression and active response induced by infectious bursal disease virus in chickens with passive antibodies. Avian Dis, 189-196.

Lumpkins, B., Batal, A., Lee, M., 2008. The effect of gender on the bacterial community in the gastrointestinal tract of broilers. Poult Sci 87, 964-967.

Maas, R.A., Venema, S., Oei, H.L., Pol, J.M., Claassen, I.J., Huurne, A.A., 2001. Efficacy of inactivated infectious bursal disease (IBD) vaccines: comparison of serology with protection of progeny chickens against IBD virus strains of varying virulence. Avian Pathol 30, 345-354.

MacDonald, T.T., Monteleone, G., 2005. Immunity, inflammation, and allergy in the gut. Science 307, 1920-1925.

Macfarlane, S., Quigley, M., Hopkins, M., Newton, D.F., Macfarlane, G., 1998. Polysaccharide degradation by human intestinal bacteria during growth under multi-substrate limiting conditions in a three-stage continuous culture system. FEMS Microbiol Ecol 26, 231-243.

Mackie, R.I., 2002. Mutualistic Fermentative Digestion in the Gastrointestinal Tract: Diversity and Evolution1. Integrative and Comparative Biology 42, 319-326.

Macpherson, A.J., Gatto, D., Sainsbury, E., Harriman, G.R., Hengartner, H., Zinkernagel, R.M., 2000. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 288, 2222-2226.

Macpherson, A.J., Harris, N.L., 2004. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 4, 478-485.

Maeda, N., Nakamura, R., Hirose, Y., Murosaki, S., Yamamoto, Y., Kase, T., Yoshikai, Y., 2009. Oral administration of heat-killed Lactobacillus plantarum L-137 enhances protection against influenza virus infection by stimulation of type I interferon production in mice. Int Immunopharmacol 9, 1122-1125.

Mahgoub, H.A., Bailey, M., Kaiser, P., 2012. An overview of infectious bursal disease. Arch Virol 157, 2047-2057.

Mahmood, M., Siddique, M., Hussain, I., Khan, A., Mansoor, M., 2006. Protection capability of recombinant plasmid DNA vaccine containing VP2 gene of very virulent infectious bursal disease virus in chickens adjuvanted with CpG oligodeoxynucleotide. Vaccine 24, 4838-4846.

Maity, H.K., Dey, S., Mohan, C.M., Khulape, S.A., Pathak, D.C., Vakharia, V.N., 2015. Protective efficacy of a DNA vaccine construct encoding the VP2 gene of infectious bursal disease and a truncated HSP70 of Mycobacterium tuberculosis in chickens. Vaccine 33, 1033-1039.

Man, S.M., 2011. The clinical importance of emerging Campylobacter species. Nature Reviews Gastroenterology and Hepatology 8, 669-685.

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., Nalin, R., Jarrin, C., Chardon, P., Marteau, P., 2006. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 55, 205-211.

Marchiando, A.M., Graham, W.V., Turner, J.R., 2010. Epithelial barriers in homeostasis and disease. Annu Rev Pathol-Mech Dis 5, 119-144.

Martínez-Torrecuadrada, J.L., Lázaro, B., Rodriguez, J.F., Casal, J.I., 2000. Antigenic properties and diagnostic potential of baculovirus-expressed infectious bursal disease viral proteins VPX and VP3. Clin Diagn Lab Immunol 7, 645-651.

Masanta, W.O., Heimesaat, M.M., Bereswill, S., Tareen, A.M., Lugert, R., Groß, U., Zautner, A.E., 2013. Modification of intestinal microbiota and its consequences for innate immune response in the pathogenesis of campylobacteriosis. Clin Dev Immunol 2013, 526860

Mazariegos, L.A., Lukert, P.D., Brown, J., 1990. Pathogenicity and immunosuppressive properties of infectious bursal disease "intermediate" strains. Avian Dis 34, 203-208.

McFerran, J., McNulty, M., McKillop, E., Connor, T., McCracken, R., Collins, D., Allan, G., 1980. Isolation and serological studies with infectious bursal disease viruses from fowl, turkeys and ducks: demonstration of a second serotype. Avian Pathog 9, 395-404.

Mead, G., 1989. Microbes of the avian cecum: types present and substrates utilized. J Exp Zool A Ecol Integr Physiol 252, 48-54.

Mertens, P.P., Jamieson, P.B., Dobos, P., 1982. In vitro RNA synthesis by infectious pancreatic necrosis virus-associated RNA polymerase. J Gen Virol 59, 47-56.

Metcalfe, D.D., Baram, D., Mekori, Y.A., 1997. Mast cells. Physiol Rev 77, 1033-1079.

Milićević, Ž., VUJIĆ, D., ISAKOVIĆ, K., MIĆIĆ, M., MILIĆEVIĆ, N.M., 1986. Involution of bursa of Fabricius in male and female chickens: a light microscopic histoquantitative study. Poult Sci 65, 2318-2323.

Millar, D.L., Naqi, S.A., 1980. Ubiquity of infectious bursal disease in East Texas backyard flocks. Poult Sci 59, 1949-1950.

Miller, H., Nawa, Y., 1979. Nippostrongylus brasiliensis: intestinal goblet-cell response in adoptively immunized rats. Exp Parasitol 47, 81-90.

Minot, S., Sinha, R., Chen, J., Li, H., Keilbaugh, S.A., Wu, G.D., Lewis, J.D., Bushman, F.D., 2011. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res 21, 1616-1625.

Mitsch, P., Zitterl-Eglseer, K., Köhler, B., Gabler, C., Losa, R., Zimpernik, I., 2004. The effect of two different blends of essential oil components on the proliferation of Clostridium perfringens in the intestines of broiler chickens. Poult Sci 83, 669-675.

Mo, C., Cao, Y., Lim, B., 2001. The in vivo and in vitro effects of chicken interferon α on infectious bursal disease virus and Newcastle disease virus infection. Avian Dis, 389-399.

Moen, B., Rudi, K., Svihus, B., Skånseng, B., 2012. Reduced spread of Campylobacter jejuni in broiler chickens by stimulating the bird's natural barriers. J Appl Microbiol 113, 1176-1183.

Moir, S., Fauci, A.S., 2009. B cells in HIV infection and disease. Nat Rev Immunol 9, 235-245.

Molloy, M.J., Grainger, J.R., Bouladoux, N., Hand, T.W., Koo, L.Y., Naik, S., Quinones, M., Dzutsev, A.K., Gao, J.-L., Trinchieri, G., 2013. Intraluminal containment of commensal outgrowth in the gut during infection-induced dysbiosis. Cell Host Microbe 14, 318-328.

Molnar, A., Hess, C., Pál, L., Wágner, L., Awad, W., Husvéth, F., Hess, M., Dublecz, K., 2015. Composition of diet modifies colonization dynamics of Campylobacter jejuni in broiler chickens. J Appl Microbiol 118, 245-254.

Moradian, A., Thorsen, J., Julian, R.J., 1990. Single and combined infections of specific-pathogen-free chickens with infectious bursal disease virus and an intestinal isolate of reovirus. Avian Dis 34, 63-72.

Motamed, N., Mayahi, M., Seifi, M., Jafari, R., 2013. Effect of infectious bursal disease virus on pathogenicity of avian influenza virus subtype H9N2 in broiler chicks. J Vet Med Anim Health 5, 276-280.

Müller, H., Islam, M.R., Raue, R., 2003. Research on infectious bursal disease--the past, the present and the future. Vet Microbiol 97, 153-165.

Müller, H., Mundt, E., Eterradossi, N., Islam, M.R., 2012. Current status of vaccines against infectious bursal disease. Avian Pathol 41, 133-139.

Müller, H., Nitschke, R., 1987. The two segments of the infectious bursal disease virus genome are circularized by a 90,000-Da protein. Virol 159, 174-177.

Müller, H., Schnitzler, D., Bernstein, F., Becht, H., Cornelissen, D., Lutticken, D.H., 1992. Infectious bursal disease of poultry: antigenic structure of the virus and control. Vet Microbiol 33, 175-183.

Müller, H., Scholtissek, C., Becht, H., 1979a. The genome of infectious bursal disease virus consists of two segments of double-stranded RNA. J Virol 31, 584-589.

Müller, R., Kaufer, I., Reinacher, M., Weiss, E., 1979b. Immunofluorescent studies of early virus propagation after oral infection with infectious bursal disease virus (IBDV). Zentralblatt fur Veterinarmedizin. Reihe B. J Vet Med. Series B 26, 345-352.

Mundt, E., Beyer, J., Müller, H., 1995. Identification of a novel viral protein in infectious bursal disease virus-infected cells. J Gen Virol 76, 437-443.

Mundt, E., Kollner, B., Kretzschmar, D., 1997. VP5 of infectious bursal disease virus is not essential for viral replication in cell culture. J Virol 71, 5647-5651.

Myszewski, M.A., Stern, N.J., 1990. Influence of Campylobacter jejuni cecal colonization on immunoglobulin response in chickens. Avian Dis, 588-594.

Nagy, N., Magyar, A., Dávid, C., Gumati, M.K., Oláh, I., 2001. Development of the follicle-associated epithelium and the secretory dendritic cell in the bursa of fabricius of the guinea fowl (Numida meleagris) studied by novel monoclonal antibodies. Anat Rec 262, 279-292.

Nagy, N., Magyar, A., Tóth, M., Oláh, I., 2004. Quail as the chimeric counterpart of the chicken: morphology and ontogeny of the bursa of Fabricius. J Morphol 259, 328-339.

Nakamura, K., Yuasa, N., Abe, H., Narita, M., 1990. Effect of infectious bursal disease virus on infections produced by Escherichia coli of high and low virulence in chickens. Avian Pathog 19, 713-721.

Naqi, S., Millar, D., 1979. Morphologic changes in the bursa of fabricius of chickens after inoculation with infectious bursal disease virus. Am J Vet Res 40, 1134-1139.

Neutra, M.R., Pringault, E., Kraehenbuhl, J.-P., 1996. Antigen sampling across epithelial barriers and induction of mucosal immune responses. Annu Rev Immunol 14, 275-300.

Noujaim, J., Filho, R.A., Lima, E., Okamoto, A.S., Amorim, R.L., Neto, R.T., 2008. Detection of T lymphocytes in intestine of broiler chicks treated with Lactobacillus spp. and challenged with Salmonella enterica serovar Enteritidis. Poult Sci 87, 927-933.

O'Hara, A.M., Shanahan, F., 2006. The gut flora as a forgotten organ. EMBO Rep 7, 688-693.

OLAH, I., GLICK, B., 1979. Structure of the germinal centers in the chicken caecal tonsil: light and electron microscopic and autoradiographic studies. Poult Sci 58, 195-210.

Oláh, I., Glick, B., 1984. Meckel's diverticulum. I. Extramedullary myelopoiesis in the yolk sac of hatched chickens (Gallus domesticus). Anat Rec 208, 243-252.

Olah, I., Glick, B., Taylor, R., 1984. Meckel's diverticulum. II. A novel lymphoepithelial organ in the chicken. Anat Rec 208, 253-263.

Oláh, I., Nagy, N., Magyar, A., Palya, V., 2003. Esophageal tonsil: a novel gut-associated lymphoid organ. Poult Sci 82, 767-770.

Oláh, I., Vervelde, L., 2008. Structure of the avian lymphoid system. In: Dacison F, Kaspers B, Schat KA, editors. Avian Immunology. London: Academic Press. pp. 13-49,

Onaga, H., Togo, M., Otaki, Y., Tajima, M., 1989. Influence of live virus vaccination against infectious bursal disease on coccidial infection in chickens. Nihon juigaku zasshi. J Vet Med Sci 51, 463-465.

Oppling, V., Müller, H., Becht, H., 1991. Heterogeneity of the antigenic site responsible for the induction of neutralizing antibodies in infectious bursal disease virus. Arch Virol 119, 211-223.

Palmquist, J.M., Khatri, M., Cha, R., Goddeeris, B., Walcheck, B., Sharma, J., 2006. In vivo activation of chicken macrophages by infectious bursal disease virus. Viral Immunol 19, 305-315.

Pan, D., Yu, Z., 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes 5, 108-119.

Pedroso, A., Menten, J., Lambais, M., Racanicci, A., Longo, F., Sorbara, J., 2006. Intestinal bacterial community and growth performance of chickens fed diets containing antibiotics. Poult Sci 85, 747-752.

Pejkovski, C., Davelaar, F.G., Kouwenhoven, B., 1979. Immunosuppressive effect of infectious bursal disease virus on vaccination against infectious bronchitis. Avian Pathol 8, 95-106.

Perlman, D., Ampel, N.M., Schifman, R.B., Cohn, D.L., Patton, C.M., Aguirre, M.L., Wang, W.-L.L., Blaser, M.J., 1988. Persistent Campylobacter jejuni infections in patients infected with human immunodeficiency virus(HIV). Ann Intern Med 108, 540-546.

Perumbakkam, S., Hunt, H.D., Cheng, H.H., 2014. Marek's disease virus influences the core gut microbiome of the chicken during the early and late phases of viral replication. FEMS Microbiol Ecol 90, 300-312.

Peters, G., 1967. The histological changes of gumboro diseases. Berl Munch Tierarztl Wochenschr 80, 394.

Phillips, R.A., Opitz, H.M., 1995. Pathogenicity and persistence of Salmonella enteritidis and egg contamination in normal and infectious bursal disease virus-infected leghorn chicks. Avian Dis 39, 778-787.

Pielsticker, C., 2013. Investigations on the humoral and cell-mediated immune response in chicken after inoculation with Campylobacter jejuni strains of human and avian origin. University of Veterinary Medicine Hannover.

Pielsticker, C., Glünder, G., Rautenschlein, S., 2016. Colonization pattern of C. jejuni isolates of human and avian origin and differences in the induction of immune responses in chicken. Vet Immunol Immunopathol 169, 1-9.

Pitcovski, J., Gutter, B., Gallili, G., Goldway, M., Perelman, B., Gross, G., Krispel, S., Barbakov, M., Michael, A., 2003. Development and large-scale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. Vaccine 21, 4736-4743.

Polansky, O., Sekelova, Z., Faldynova, M., Sebkova, A., Sisak, F., Rychlik, I., 2015. Characterisation of the most important metabolic pathways and biological processes expressed in chicken caecal microbiota. Appl Environ Microbiol, AEM. 03473-03415.

Pourabedin, M., Guan, L., Zhao, X., 2015. Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. Microbiome 3, 15.

Pradhan, S.N., Prince, P.R., Madhumathi, J., Arunkumar, C., Roy, P., Narayanan, R.B., Antony, U., 2014. DNA vaccination with VP2 gene fragment confers protection against Infectious Bursal Disease Virus in chickens. Vet Microbiol 171, 13-22.

Prandini, F., Simon, B., Jung, A., Poppel, M., Lemiere, S., Rautenschlein, S., 2016. Comparison of infectious bursal disease live vaccines and a HVT-IBD vector vaccine and their effects on the immune system of commercial layer pullets. Avian Pathol 45, 114-125.

Qi, X., Chen, Y., Ren, X., Zhang, L., Gao, L., Wang, N., Qin, L., Wang, Y., Gao, Y., Wang, X., 2014. A reassortment vaccine candidate as the improved formulation to induce protection against very virulent infectious bursal disease virus. Vaccine 32, 1436-1443.

Qin, L., Qi, X., Gao, Y., Gao, H., Lu, X., Wang, Y., Bu, Z., Wang, X., 2010. VP5-deficient mutant virus induced protection against challenge with very virulent infectious bursal disease virus of chickens. Vaccine 28, 3735-3740.

Qu, A., Brulc, J.M., Wilson, M.K., Law, B.F., Theoret, J.R., Joens, L.A., Konkel, M.E., Angly, F., Dinsdale, E.A., Edwards, R.A., 2008. Comparative metagenomics reveals host specific metavirulomes and horizontal gene transfer elements in the chicken cecum microbiome. PloS one 3, e2945.

Qureshi, M., Hussain, I., Heggen, C., 1998. Understanding Immunol in disease development and control. Poult Sci 77, 1126-1129.

Ragland, W.L., Novak, R., El-Attrache, J., Savic, V., Ester, K., 2002. Chicken anemia virus and infectious bursal disease virus interfere with transcription of chicken IFN-alpha and IFN-gamma mRNA. J Interferon Cytokine Res 22, 437-441.

Ramirez-Nieto, G., Shivaprasad, H., Kim, C.-H., Lillehoj, H.S., Song, H., Osorio, I.G., Perez, D.R., 2010. Adaptation of a mallard H5N2 low pathogenicity influenza virus in chickens with prior history of infection with infectious bursal disease virus. Avian Dis 54, 513-521.

Rasoli, M., Yeap, S.K., Tan, S.W., Roohani, K., Kristeen-Teo, Y.W., Alitheen, N.B., Rahaman, Y.A., Aini, I., Bejo, M.H., Kaiser, P., 2015. Differential modulation of immune response and cytokine profiles in the bursae and spleen of chickens infected with very virulent infectious bursal disease virus. BMC Vet Res 11, 1.

Ratcliffe, M.J., Lassila, O., Pink, J.R.L., Vainio, O., 1986. Avian B cell precursors: Surface immunoglobulin expression is an early, possibly bursa-independent event. Eur J Immunol 16, 129-133.

Rauf, A., Khatri, M., Murgia, M.V., Jung, K., Saif, Y.M., 2011a. Differential modulation of cytokine, chemokine and Toll like receptor expression in chickens infected with classical and variant infectious bursal disease virus. Vet Res 42, 85.

Rauf, A., Khatri, M., Murgia, M.V., Saif, Y.M., 2011b. Expression of perforin–granzyme pathway genes in the bursa of infectious bursal disease virus-infected chickens. Dev Comp Immunol 35, 620-627.

Rauf, A., Khatri, M., Murgia, M.V., Saif, Y.M., 2012. Fas/FasL and perforin-granzyme pathways mediated T cell cytotoxic responses in infectious bursal disease virus infected chickens. Results Immunol 2, 112-119.

Rautenschlein, S., Kraemer, C., Montiel, E., Vanmarcke, J., Haase, C., 2007. Bilateral effects of vaccination against infectious bursal disease and Newcastle disease in specific-pathogen-free layers and commercial broiler chickens. Avian Dis 51, 14-20.

Rautenschlein, S., Kraemer, C., Vanmarcke, J., Montiel, E., 2005. Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. Avian Dis 49, 231-237.

Rautenschlein, S., von Samson-Himmelstjerna, G., Haase, C., 2007. A comparison of immune responses to infection with virulent infectious bursal disease virus (IBDV) between specific-pathogen-free chickens infected at 12 and 28 days of age. Vet Immunol Immunopathol 115, 251-260.

Rautenschlein, S., Yeh, H.-Y., Njenga, M., Sharma, J., 2002. Role of intrabursal T cells in infectious bursal disease virus (IBDV) infection: T cells promote viral clearance but delay follicular recovery. Arch Virol 147, 285-304.

Rauw, F., Lambrecht, B., Van den Berg, T., 2007. Pivotal role of ChIFNγ in the pathogenesis and immunosuppression of infectious bursal disease. Avian Pathog 36, 367-374.

Reddy, S., Silim, A., Ratcliffe, M., 1992. Biological roles of the major capsid proteins and relationships between the two existing serotypes of infectious bursal disease virus. Arch Virol 127, 209-222.

Rehman, H.U., Vahjen, W., Awad, W.A., Zentek, J., 2007. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Arch Anim Nutr 61, 319-335.

Rehman, Z., Meng, C., Umar, S., Munir, M., Ding, C., 2016. Interaction of infectious bursal disease virus with the immune system of poultry. Worlds Poult Sci J 72, 805-820.

Reynaud, C.-A., Mackay, C.R., Müller, R.G., Weill, J.-C., 1991. Somatic generation of diversity in a mammalian primary lymphoid organ: the sheep ileal Peyer's patches. Cell 64, 995-1005.

Robyn, J., Rasschaert, G., Messens, W., Pasmans, F., Heyndrickx, M., 2012. Screening for lactic acid bacteria capable of inhibiting Campylobacter jejuni in in vitro simulations of the broiler chicken caecal environment. Benef Microbes 3, 299-308.

Rodenberg, J., Sharma, J., Belzer, S., Nordgren, R., Naqi, S., 1994. Flow cytometric analysis of B cell and T cell subpopulations in specific-pathogen-free chickens infected with infectious bursal disease virus. Avian Dis, 16-21.

Rodríguez-Lecompte, J.C., Niño-Fong, R., Lopez, A., Markham, R.F., Kibenge, F.S., 2005. Infectious bursal disease virus (IBDV) induces apoptosis in chicken B cells. Comp Immunol Microbiol Infect Dis 28, 321-337.

Rodríguez, M.L., Rebolé, A., Velasco, S., Ortiz, L.T., Treviño, J., Alzueta, C., 2012. Wheat-and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microbiota. J Sci Food Agric 92, 184-190.

Röll, S., Härtle, S., Lütteke, T., Kaspers, B., Härtle, S., 2017. Tissue and time specific expression pattern of interferon regulated genes in the chicken. BMC Genomics 18, 264.

Rong, J., Cheng, T., Liu, X., Jiang, T., Gu, H., Zou, G., 2005. Development of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. Vaccine 23, 4844-4851.

Rosenberger, J., Cloud, S., Metz, A. 1987. Use of infectious bursal disease virus variant vaccines in broilers and broiler breeders. In: Proceedings-WPDC (USA).pp, 105-106

Rosenberger, J., Gelb Jr, J., 1978. Response to several avian respiratory viruses as affected by infectious bursal disease virus. Avian Dis, 95-105.

Round, J.L., Mazmanian, S.K., 2009. The gut microbiome shapes intestinal immune responses during health and disease. Nat Rev Immunol 9, 313.

Saengkerdsub, S., Anderson, R.C., Wilkinson, H.H., Kim, W.-K., Nisbet, D.J., Ricke, S.C., 2007. Identification and quantification of methanogenic archaea in adult chicken ceca. Appl Environ Microbiol 73, 353-356.

Sahin, O., Luo, N., Huang, S., Zhang, Q., 2003. Effect of Campylobacter-specific maternal antibodies on Campylobacter jejuni colonization in young chickens. Appl Environ Microbiol 69, 5372-5379.

Saif, Y., 1998. Infectious bursal disease and hemorrhagic enteritis. Poult Sci 77, 1186-1189.

Saif, Y.M., 1991. Immunosuppression induced by infectious bursal disease virus. Vet Immunol Immunopathol 30, 45-50.

Salanitro, J., Fairchilds, I., Zgornicki, Y., 1974. Isolation, culture characteristics, and identification of anaerobic bacteria from the chicken cecum. Appl Microbiol 27, 678-687.

Sanchez-Refusta, F., Ciriaco, E., Germana, A., Germanà, G., Vega, J., 1996. Age-related changes in the medullary reticular epithelial cells of the pigeon bursa of fabricius. Anat Rec 246, 473-480.

Santivatr, D., Maheswaran, S., Newman, J., Pomeroy, B., 1981. Effect of infectious bursal disease virus infection on the phagocytosis of Staphylococcus aureus by mononuclear phagocytic cells of susceptible and resistant strains of chickens. Avian Dis, 303-311.

Sanyal, S., Islam, K., Neogy, P., Islam, M., Speelman, P., Huq, M., 1984. Campylobacter jejuni diarrhea model in infant chickens. Infect Immun 43, 931-936.

Sayegh, C.E., Demaries, S.L., Pike, K.A., Friedman, J.E., Ratcliffe, M.J., 2000. The chicken B-cell receptor complex and its role in avian B-cell development. Immunol Rev 175, 187-200.

Schat, K., Myers, T., 1991. Avian intestinal immunity. Critical Reviews in Poultry Biology (United Kingdom).

Schijns, V., Sharma, J., Tarpey, I., Davison, F., Kaspers, B., Schat, K., 2008. Practical aspects of poultry vaccination. Avian Immunol 1, 373-393.

Schijns, V.E., Weining, K.C., Nuijten, P., Rijke, E.O., Staeheli, P., 2000. Immunoadjuvant activities of E. coli- and plasmid-expressed recombinant chicken IFN-α/β, IFN-γ and IL-1β in 1-day-and 3-week-old chickens. Vaccine 18, 2147-2154.

Schwarz, A., Gauly, M., Abel, H., Daş, G., Humburg, J., Rohn, K., Breves, G., Rautenschlein, S., 2011. Immunopathogenesis of Ascaridia galli infection in layer chicken. Dev Comp Immunol 35, 774-784.

Sekelja, M., Rud, I., Knutsen, S., Denstadli, V., Westereng, B., Naes, T., Rudi, K., 2012. Abrupt temporal fluctuations in the chicken fecal microbiota are explained by its gastrointestinal origin. Appl Environ Microbiol 78, 2941-2948.

Shakouri, M., Iji, P., Mikkelsen, L., Cowieson, A., 2009. Intestinal function and gut microbiota of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J Anim Physiol Anim Nutr (Berl) 93, 647-658.

Shanker, S., Lee, A., Sorrell, T., 1990. Horizontal transmission of Campylobacter jejuni amongst broiler chicks: experimental studies. Epidemiology and infection 104, 101-110.

Sharma, J., 2003. The avian immune system. Diseases of Poultry. YM Saif, ed. Iowa State University Press, Ames, 5-16.

Sharma, J., Dohms, J., Metz, A., 1989. Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of IBDV and their effect on humoral and cellular immunocompetence of SPF chickens. Avian Dis 33, 112-124.

Sharma, J., Lee, L., 1983. Effect of infectious bursal disease on natural killer cell activity and mitogenic response of chicken lymphoid cells: role of adherent cells in cellular immune suppression. Infect Immun 42, 747-754.

Sharma, J.M., 1984. Effect of infectious bursal disease virus on protection against Marek's disease by turkey herpesvirus vaccine. Avian Dis 28, 629-640.

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.

Shaufi, M.A.M., Sieo, C.C., Chong, C.W., Gan, H.M., Ho, Y.W., 2015. Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses. Gut Pathog 7, 4.

Sheridan, B.S., Lefrançois, L., 2010. Intraepithelial lymphocytes: to serve and protect. Curr Gastroenterol Rep 12, 513-521.

Shira, E.B., Sklan, D., Friedman, A., 2005. Impaired immune responses in broiler hatchling hindgut following delayed access to feed. Vet Immunol Immunopathol 105, 33-45.

Sivanandan, V., Maheswaran, S., 1980. Immune profile of infectious bursal disease: I. Effect of infectious bursal disease virus on peripheral blood T and B lymphocytes of chickens. Avian Dis, 715-725.

Skånseng, B., Kaldhusdal, M., Moen, B., Gjevre, A.G., Johannessen, G., Sekelja, M., Trosvik, P., Rudi, K., 2010. Prevention of intestinal Campylobacter jejuni colonization in broilers by combinations of in-feed organic acids. J Appl Microbiol 109, 1265-1273.

Skånseng, B., Kaldhusdal, M., Rudi, K., 2006. Comparison of chicken gut colonisation by the pathogens Campylobacter jejuni and Clostridium perfringens by real-time quantitative PCR. Mol Cell Probes 20, 269-279.

Smith, A.L., Beal, R., 2008. The avian enteric immune system in health and disease. Avian Immunol 1, 243-271.

Smith, C.K., AbuOun, M., Cawthraw, S.A., Humphrey, T.J., Rothwell, L., Kaiser, P., Barrow, P.A., Jones, M.A., 2008. Campylobacter colonization of the chicken induces a proinflammatory response in mucosal tissues. FEMS Immunol Med Microbiol 54, 114-121.

Smith, C.K., Kaiser, P., Rothwell, L., Humphrey, T., Barrow, P.A., Jones, M.A., 2005. Campylobacter jejuni-induced cytokine responses in avian cells. Infect Immun 73, 2094-2100.

Smith, J., Sadeyen, J.-R., Butter, C., Kaiser, P., Burt, D.W., 2015. Analysis of the early immune response to infection by infectious bursal disease virus in chickens differing in their resistance to the disease. J Virol 89, 2469-2482.

Snyder, D., Vakharia, V., Savage, P., 1992. Naturally occurring-neutralizing monoclonal antibody escape variants define the epidemiology of infectious bursal disease viruses in the United States. Arch Virol 127, 89-101.

Sobhani, I., Tap, J., Roudot-Thoraval, F., Roperch, J.P., Letulle, S., Langella, P., Corthier, G., Van Nhieu, J.T., Furet, J.P., 2011. Microbial dysbiosis in colorectal cancer (CRC) patients. PloS one 6, e16393.

Sofka, D., Pfeifer, A., Gleiß, B., Paulsen, P., Hilbert, F., 2015. Changes within the intestinal flora of broilers by colonisation with Campylobacter jejuni. Berl. Münch. Tierärztl. Wochenschr 128, 104-110.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L.G., Gratadoux, J.-J., Blugeon, S., Bridonneau, C., Furet, J.-P., Corthier, G., 2008. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci 105, 16731-16736.

Specian, R.D., Oliver, M.G., 1991. Functional biology of intestinal goblet cells. American Am J Physiol Cell Physiol 260, C183-C193.

Spies, U., Müller, H., 1990. Demonstration of enzyme activities required for cap structure formation in infectious bursal disease virus, a member of the birnavirus group. J Gen Virol 71, 977-981.

Spits, H., Di Santo, J.P., 2011. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. Nat Immunol 12, 21-27.

Stanley, D., Denman, S.E., Hughes, R.J., Geier, M.S., Crowley, T.M., Chen, H., Haring, V.R., Moore, R.J., 2012a. Intestinal microbiota associated with differential feed conversion efficiency in chickens. Appl Microbiol Biotechnol 96, 1361-1369.

Stanley, D., Keyburn, A.L., Denman, S.E., Moore, R.J., 2012b. Changes in the caecal microbiota of chickens following Clostridium perfringens challenge to induce necrotic enteritis. Vet Microbiol 159, 155-162.

Stappenbeck, T.S., Hooper, L.V., Gordon, J.I., 2002. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci 99, 15451-15455.

Stas, T., Jordan, F., Woldehiwet, Z., 1999. Experimental infection of chickens with Campylobacter jejuni: strains differ in their capacity to colonize the intestine. Avian Pathog 28, 61-64.

Stojanov, I., Milić, N., Ašanin, R., Vidić, B., Nišavić, J., Grgić, Ž., Prica, N., 2008. Effects of some immunosuppressive factors on campylobacteriosis outbreaks in poultry. Acta veterinaria 58, 167-177.

Subler, K.A., Mickael, C.S., Jackwood, D.J., 2006. Infectious bursal disease virus-induced immunosuppression exacerbates Campylobacter jejuni colonization and shedding in chickens. Avian Dis 50, 179-184.

Sultan, S., Osman, N., Mohamed, M.A., Ahmed, A.I., Abdallah, R.A., Faraouk, M., Taha, M., 2016. Infectious bursal disease vaccine ameliorates velogenic Newcastle disease virus infection in immunopotentiated chickens. Comp Clin Path 25, 91-100.

Sun, Q., Shang, Y., She, R., Jiang, T., Wang, D., Ding, Y., Yin, J., 2013. Detection of intestinal intraepithelial lymphocytes, goblet cells and secretory IgA in the intestinal mucosa during Newcastle disease virus infection. Avian Pathog 42, 541-545.

Survashe, B., Aitken, I., Powell, J., 1979. The response of the Harderian gland of the fowl to antigen given by the ocular route i. Histological changes. Avian Pathog 8, 77-93.

Tacken, M.G., Peeters, B.P., Thomas, A.A., Rottier, P.J., Boot, H.J., 2002. Infectious bursal disease virus capsid protein VP3 interacts both with VP1, the RNA-dependent RNA polymerase, and with viral double-stranded RNA. J Virol 76, 11301-11311.

Tanimura, N., Sharma, J., 1997. Appearance of T cells in the bursa of Fabricius and cecal tonsils during the acute phase of infectious bursal disease virus infection in chickens. Avian Dis, 638-645.

Tanimura, N., Sharma, J.M., 1998. In-situ apoptosis in chickens infected with infectious bursal disease virus.J Comp Pathol 118, 15-27.

Tanimura, N., Tsukamoto, K., Nakamura, K., Narita, M., Maeda, M., 1995. Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunohistochemistry. Avian Dis, 9-20.

Teirlynck, E., Bjerrum, L., Eeckhaut, V., Huygebaert, G., Pasmans, F., Haesebrouck, F., Dewulf, J., Ducatelle, R., Van Immerseel, F., 2009. The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. Br J Nurtr 102, 1453-1461.

Thibodeau, A., Fravalo, P., Yergeau, E., Arsenault, J., Lahaye, L., Letellier, A., 2015. Chicken Caecal Microbiome Modifications Induced by Campylobacter jejuni Colonization and by a Non-Antibiotic Feed Additive. PLoS One 10, e0131978.

Thompson, G., Mohammed, H., Bauman, B., Naqi, S., 1997. Systemic and local antibody responses to infectious bronchitis virus in chickens inoculated with infectious bursal disease virus and control chickens. Avian Dis, 519-527.

Tippenhauer, M., Heller, D.E., Weigend, S., Rautenschlein, S., 2013. The host genotype influences infectious bursal disease virus pathogenesis in chickens by modulation of T cells responses and cytokine gene expression. Dev Comp Immunol 40, 1-10.

Toro, H., Van Santen, V., Hoerr, F., Breedlove, C., 2009. Effects of chicken anemia virus and infectious bursal disease virus in commercial chickens. Avian Dis 53, 94-102.

Torok, V.A., Allison, G.E., Percy, N.J., Ophel-Keller, K., Hughes, R.J., 2011. Influence of antimicrobial feed additives on broiler commensal posthatch gut microbiota development and performance. Appl Environ Microbiol 77, 3380-3390.

Tsukamoto, K., Matsumura, T., Mase, M., Imai, K., 1995. A highly sensitive, broad-spectrum infectivity assay for infectious bursal disease virus. Avian Dis, 575-586.

Tsukamoto, K., Tanimura, N., Hihara, H., Shirai, J., Imai, K., Nakamura, K., Maeda, M., 1992. Isolation of virulent infectious bursal disease virus from field outbreaks with high mortality in Japan. J Vet Med Sci 54, 153-155.

Udayamputhoor, R.S., Hariharan, H., Van Lunen, T.A., Lewis, P.J., Heaney, S., Price, L., Woodward, D., 2003. Effects of diet formulations containing proteins from different sources on intestinal colonization by Campylobacter jejuni in broiler chickens. Can J Vet Res 67, 204-212.

Uni, Z., 1999. Functional development of the small intestine in domestic birds: Cellular and molecular aspects. Poultry and Avian Biology Reviews 10, 167-179.

Uni, Z., Noy, Y., Sklan, D., 1999. Posthatch development of small intestinal function in the poult. Poult Sci 78, 215-222.

Uni, Z., Tako, E., Gal-Garber, O., Sklan, D., 2003. Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poult Sci 82, 1747-1754.

Ursula, I., Gorbalenya, A.E., Schirrmeier, H., Behrens, S.-E., Letzel, T., Mundt, E., 2004. VP1 of infectious bursal disease virus is an RNA-dependent RNA polymerase. J Gen Virol 85, 2221-2229.

Vaezirad, M.M., Keestra-Gounder, A.M., de Zoete, M.R., Koene, M.G., Wagenaar, J.A., van Putten, J.P., 2016. Invasive behavior of Campylobacter jejuni in immunosuppressed chicken. Virulence, 1-13.

Vakharia, V.N., He, J., Ahamed, B., Snyder, D.B., 1994. Molecular basis of antigenic variation in infectious bursal disease virus. Virus Res 31, 265-273.

Van den Berg, T., Gonze, M., Morales, D., Meulemans, G., 1996. Acute infectious bursal disease in poultry: immunological and molecular basis of antigenicity of a highly virulent strain. Avian Pathog 25, 751-768.

Van den Berg, T., Morales, D., Eterradossi, N., Rivallan, G., Toquin, D., Raue, R., Zierenberg, K., Zhang, M., Zhu, Y., Wang, C., 2004. Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. Avian Pathog 33, 470-476.

Van den Berg, T., Ona, A., Morales, D., Rodriguez, J. 2001. Experimental inoculation of game/ornamental birds with a very virulent strain of IBDV. In: International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia, 236-246.

van den Berg, T.P., Eterradossi, N., Toquin, D., Meulemans, G., 2000. Infectious bursal disease (Gumboro disease). Rev Sci Tech 19, 509-543.

Van der Hoeven-Hangoor, E., Van Der Vossen, J., Schuren, F., Verstegen, M., De Oliveira, J., Montijn, R., Hendriks, W., 2013. Ileal microbiota composition of broilers fed various commercial diet compositions. Poult Sci 92, 2713-2723.

Van Deun, K., Haesebrouck, F., Van Immerseel, F., Ducatelle, R., Pasmans, F., 2008a. Short-chain fatty acids and L-lactate as feed additives to control Campylobacter jejuni infections in broilers. Avian Pathol 37, 379-383.

Van Deun, K., Pasmans, F., Ducatelle, R., Flahou, B., Vissenberg, K., Martel, A., Van den Broeck, W., Van Immerseel, F., Haesebrouck, F., 2008b. Colonization strategy of Campylobacter jejuni results in persistent infection of the chicken gut. Vet Microbiol 130, 285-297.

Van Deun, K., Pasmans, F., Van Immerseel, F., Ducatelle, R., Haesebrouck, F., 2008c. Butyrate protects Caco-2 cells from Campylobacter jejuni invasion and translocation. Br J Nurtr 100, 480-484.

Van Gerwe, T., Bouma, A., Klinkenberg, D., Wagenaar, J.A., Jacobs-Reitsma, W.F., Stegeman, A., 2010. Medium chain fatty acid feed supplementation reduces the probability of Campylobacter jejuni colonization in broilers. Vet Microbiol 143, 314-318.

van Gerwe, T., Miflin, J.K., Templeton, J.M., Bouma, A., Wagenaar, J.A., Jacobs-Reitsma, W.F., Stegeman, A., Klinkenberg, D., 2009. Quantifying transmission of Campylobacter jejuni in commercial broiler flocks. Appl Environ Microbiol 75, 625-628.

Varmuzova, K., Kubasova, T., Davidova-Gerzova, L., Sisak, F., Havlickova, H., Sebkova, A., Faldynova, M., Rychlik, I., 2016. Composition of gut microbiota influences resistance of newly hatched chickens to Salmonella Enteritidis infection. Front Microbiol 7.

Vasconcelos, A., Lam, K., 1995. Apoptosis in chicken embryos induced by the infectious bursal disease virus.J Comp Pathol 112, 327-338.

Vervelde, L., Davison, T., 1997. Comparison of the in situ changes in lymphoid cells during infection with infectious bursal disease virus in chickens of different ages. Avian Pathog 26, 803-821.

Videnska, P., Rahman, M.M., Faldynova, M., Babak, V., Matulova, M.E., Prukner-Radovcic, E., Krizek, I., Smole-Mozina, S., Kovac, J., Szmolka, A., 2014. Characterization of egg laying hen and broiler fecal microbiota in poultry farms in Croatia, Czech Republic, Hungary and Slovenia. PLoS One 9, e110076.

Vindigni, S.M., Zisman, T.L., Suskind, D.L., Damman, C.J., 2016. The intestinal microbiome, barrier function, and immune system in inflammatory bowel disease: a tripartite pathophysiological circuit with implications for new therapeutic directions. Therap Adv Gastroenterol 9, 606-625.

von Einem, U.I., Gorbalenya, A.E., Schirrmeier, H., Behrens, S.E., Letzel, T., Mundt, E., 2004. VP1 of infectious bursal disease virus is an RNA-dependent RNA polymerase. J Gen Virol 85, 2221-2229.

Vujkovic-Cvijin, I., Dunham, R.M., Iwai, S., Maher, M.C., Albright, R.G., Broadhurst, M.J., Hernandez, R.D., Lederman, M.M., Huang, Y., Somsouk, M., 2013. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med 5, 193ra191.

Waite, D.W., Taylor, M.W., 2014. Characterizing the avian gut microbiota: membership, driving influences, and potential function. Front Microbiol 5, 223.

Wang, D., Liu, Y., She, R., Xu, J., Liu, L., Xiong, J., Yang, Y., Sun, Q., Peng, K., 2009a. Reduced mucosal injury of SPF chickens by mast cell stabilization after infection with very virulent infectious bursal disease virus. Vet Immunol Immunopathol 131, 229-237.

Wang, D., Xiong, J., She, R., Liu, L., Zhang, Y., Luo, D., Li, W., Hu, Y., Wang, Y., Zhang, Q., 2008. Mast cell mediated inflammatory response in chickens after infection with very virulent infectious bursal disease virus. Vet Immunol Immunopathol 124, 19-28.

Wang, D., Zhou, X., She, R., Xiong, J., Sun, Q., Peng, K., Liu, L., Liu, Y., 2009b. Impaired intestinal mucosal immunity in specific-pathogen-free chickens after infection with very virulent infectious bursal disease virus. Poult Sci 88, 1623-1628.

Wang, S., Hibberd, M.L., Pettersson, S., Lee, Y.K., 2014. Enterococcus faecalis from healthy infants modulates inflammation through MAPK signaling pathways. PloS one 9, e97523.

Wang, X., Jiang, P., Deen, S., Wu, J., Liu, X., Xu, J., 2003. Efficacy of DNA vaccines against infectious bursal disease virus in chickens enhanced by coadministration with CpG oligodeoxynucleotide. Avian Dis 47, 1305-1312.

Wei, L., Hou, L., Zhu, S., Wang, J., Zhou, J., Liu, J., 2011. Infectious bursal disease virus activates the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway by interaction of VP5 protein with the p85α subunit of PI3K. Virol 417, 211-220.

Wei, S., Morrison, M., Yu, Z., 2013. Bacterial census of poultry intestinal microbiome. Poult Sci 92, 671-683.

Welkos, S., 1984. Experimental gastroenteritis in newly-hatched chicks infected with Campylobacter jejuni. J Med Microbiol 18, 233-248.

Whitfill, C., Haddad, E., Ricks, C., Skeeles, J., Newberry, L., Beasley, J., Andrews, P., Thoma, J., Wakenell, P., 1995. Determination of optimum formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal disease antibody with IBDV. Avian Dis, 687-699.

Wielen, P., Keuzenkamp, D., Lipman, L.v., Knapen, F., Biesterveld, S., 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microb Ecol 44, 286-293.

Williams, A., Davison, T., 2005. Enhanced immunopathology induced by very virulent infectious bursal disease virus. Avian Pathog 34, 4-14.

Williams, L., Sait, L., Trantham, E., Cogan, T., Humphrey, T., 2013. Campylobacter infection has different outcomes in fast-and slow-growing broiler chickens. Avian Dis 57, 238-241.

Willing, B., Halfvarson, J., Dicksved, J., Rosenquist, M., Järnerot, G., Engstrand, L., Tysk, C., Jansson, J.K., 2009. Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. Inflamm Bowel Dis 15, 653-660.

Winterfield, R.W., Fadly, A.M., Bickford, A., 1972. Infectivity and distribution of infectious bursal disease virus in the chicken. Persistence of the virus and lesions. Avian Dis 16, 622-632.

Withers, D.R., Davison, T.F., Young, J.R., 2006. Diversified bursal medullary B cells survive and expand independently after depletion following neonatal infectious bursal disease virus infection. Immunol 117, 558-565.

Withers, D.R., Young, J.R., Davison, T.F., 2005. Infectious bursal disease virus-induced immunosuppression in the chick is associated with the presence of undifferentiated follicles in the recovering bursa. Viral Immunol 18, 127-137.

Wong, R.T., Hon, C.C., Zeng, F., Leung, F.C., 2007. Screening of differentially expressed transcripts in infectious bursal disease virus-induced apoptotic chicken embryonic fibroblasts by using cDNA microarrays. J Gen Virol 88, 1785-1796.

Wu, S.-B., Stanley, D., Rodgers, N., Swick, R.A., Moore, R.J., 2014. Two necrotic enteritis predisposing factors, dietary fishmeal and Eimeria infection, induce large changes in the caecal microbiota of broiler chickens. Vet Microbiol 169, 188-197.

Wu, Y., Hong, L., Ye, J., Huang, Z., Zhou, J., 2009. The VP5 protein of infectious bursal disease virus promotes virion release from infected cells and is not involved in cell death. Arch Virol 154, 1873-1882.

Wyeth, P., 1975. Effect of infectious bursal disease on the response of chickens to S typhimurium and E coli infections. Vet Rec 96, 238-243.

Xiao, Y., Xiang, Y., Zhou, W., Chen, J., Li, K., Yang, H., 2016. Microbial community mapping in intestinal tract of broiler chicken. Poult Sci, pew372.

Xu, X.-G., Tong, D.-W., Wang, Z.S., Zhang, Q., Li, Z.-C., Zhang, K., Li, W., Liu, H.-J., 2011. Baculovirus virions displaying infectious bursal disease virus VP2 protein protect chickens against infectious bursal disease virus infection. Avian Dis 55, 223-229.

Yao, K., Goodwin, M.A., Vakharia, V.N., 1998. Generation of a mutant infectious bursal disease virus that does not cause bursal lesions. J Virol 72, 2647-2654.

Yasmin, A.R., Yeap, S.K., Hair-Bejo, M., Omar, A.R., 2016. Characterization of Chicken Splenic-Derived Dendritic Cells Following Vaccine and Very Virulent Strains of Infectious Bursal Disease Virus Infection. Avian Dis 60, 739-751.

Ye, C., Jia, L., Sun, Y., Hu, B., Wang, L., Lu, X., Zhou, J., 2014. Inhibition of antiviral innate immunity by birnavirus VP3 protein via blockage of viral double-stranded RNA binding to the host cytoplasmic RNA detector MDA5. J Virol 88, 11154-11165.

Yegani, M., Korver, D., 2008. Factors affecting intestinal health in poultry. Poult Sci 87, 2052-2063.

Yeoman, C.J., Chia, N., Jeraldo, P., Sipos, M., Goldenfeld, N.D., White, B.A., 2012. The microbiome of the chicken gastrointestinal tract. Anim Health Res Rev 13, 89-99.

Yin, Y., Lei, F., Zhu, L., Li, S., Wu, Z., Zhang, R., Gao, G.F., Zhu, B., Wang, X., 2010. Exposure of different bacterial inocula to newborn chicken affects gut microbiota development and ileum gene expression. ISME J 4, 367-376.

Yogasundram, K., Shane, S.M., Harrington, K.S., 1989. Prevalence of *Campylobacter jejuni* in selected domestic and wild birds in Louisiana. Avian Dis 33, 664-667.

Yurong, Y., Ruiping, S., ShiMin, Z., Yibao, J., 2005. Effect of probiotics on intestinal mucosal immunity and ultrastructure of cecal tonsils of chickens. Arch Anim Nutr 59, 237-246.

Zhang, H.-h., Yang, X.-m., Xie, Q.-m., Ma, J.-y., Luo, Y.-n., Cao, Y.-c., Chen, F., Bi, Y.-z., 2010. The potent adjuvant effects of chicken β-defensin-1 when genetically fused with infectious bursal disease virus VP2 gene. Vet Immunol Immunopathol 136, 92-97.

Zhao, L., Wang, G., Siegel, P., He, C., Wang, H., Zhao, W., Zhai, Z., Tian, F., Zhao, J., Zhang, H., 2013. Quantitative genetic background of the host influences gut microbiomes in chickens. Sci Rep 3, 1163.

Zhu, X.Y., Zhong, T., Pandya, Y., Joerger, R.D., 2002. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl Environ Microbiol 68, 124-137.

Zierenberg, K., Raue, R., Müller, H., 2001. Rapid identification of" very virulent" strains of infectious bursal disease virus by reverse transcription-polymerase chain reaction combined with restriction enzyme analysis. Avian Pathog 30, 55-62.

Zoetendal, E.G., Akkermans, A.D., De Vos, W.M., 1998. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl Environ Microbiol 64, 3854-3859.

10. Appendix

10.1. Declaration

I herewith declare that I autonomously carried out the PhD-thesis entitled

“Interference of infectious bursal disease virus with the development of the gut-associated immune system and the establishment of the gut microbiota”.

No third party assistance has been used.

I did not receive any assistance in return for payment by consulting agencies or any other person. No one received any kind of payment for direct or indirect assistance in correlation to the content of the submitted thesis.

I conducted the project at the following institution(s)*: Clinic for Poultry, University of Veterinary Medicine Hannover, Hannover, Germany.

The thesis has not been submitted elsewhere for an exam, as thesis or forevaluation in a similar context.

I hereby affirm the above statements to be complete and true to the best of my knowledge.

_____

[date], signature

10.2. Acknowledgments

First of all, I am particularly grateful to Prof. Dr. Silke Rautenschlein for giving me this opportunity to study in Germany, and for giving me this fantastic topic. Thank you for your continuous advice, encouragement, and supervision throughout the research and Ph.D. study as well as in daily life.

I would like to thank my co-supervisors, Prof. Dr. Gorg Herrler and Prof. Dr. Reimar Johne. Thank you for sharing your ideas and suggestions as well as for the valuable discussions during our thesis evaluation meetings.

I am very grateful to all my colleagues for the help and camaraderie, both during our experiment and in daily life. I would also like to thank Dr. Lydia Teske, Dr. Henning Peterson, Dr. Zifeng Han, Dr. Hicham Sid, Thomas Willer, Marina Dobner, Rebecca Lindenwald, Rebecca Knab and Dr. Annette Kaiser,for sharing their knowledge as well as for their help, both in work and in life.

Furthermore, I would like to thank the entire membership of the Clinic for Poultry. It is a great pleasure for me to work with all of you. Thank you for all you have done for me.

Special thanks to Sonja Bernhard, Katja Stolpe, Sabrina Techel, and Hans Bertram, who provided much assistance in caring for the birds throughout my experiments. Further, I thank Hilke Barthels, Christine Haase, Rita Leise, and Carola Pastoors for their technical support in the laboratory, not only during my animal experiments but also during the preparation step.

Additionally, I would like to express my gratefulness to Dr. Lydia Teske, Dr. Henning Peterson, Dr. Zifeng Han, Dr. Thomas Willer, Ms. Marina Dobner, who helped me throughout each necropsy day during my animal experiments. Thank Rebecca Lindenwald for providing the wonderful pictures presented in the literature review.

Special thanks to Prof. Dr. Ulrich Neumann. Thank you for your recommendation, and for giving me such a wonderful chance to study in Germany, and to build a strong scientific bridge between China and Germany.

Thank you to the China Scholarship Council (CSC) for providing me financial support during my studies in Germany.

I also would like to thank all of my Chinese friends in Hannover, who provided help and support in the face of all the hardships I have encountered during the past few years.

In particular, I would like to thank my beloved husband Jannik Godzik for his understanding, quiet patience, and unwavering and constant love. Thanks to him for shaping my positive attitude toward life and for illuminating the darkness of my mind. His whole-hearted backup and encouragement was, in the end, what made this dissertation possible.

Finally, I would like to thank my entire family—my beloved parents and my brother—for always being there to love me. Especially my mother: thank you for keeping me calm when I was stressed and felt lost, and encouraging me to go on through the darkness.