Research in Veterinary Science 108 (2016) 54–59

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Circulating strains of variant infectious bursal disease virus may pose a challenge for antibiotic-free chicken farming in Canada

Shanika Kurukulsuriya [a], Khawaja Ashfaque Ahmed [a], Davor Ojkic [b], Thushari Gunawardana [a], Ashish Gupta [a], Kalhari Goonewardene [a], Ruwani Karunaratne [a], Shelly Popowich [a], Philip Willson [c], Suresh K. Tikoo [d,e], Susantha Gomis [a,*]

[a] Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, S7N 5B4, SK, Canada
[b] Animal Health Laboratory, University of Guelph, P.O. Box 3612, Guelph, ON N1H 6R8, Canada
[c] Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, S7N 5E5, SK, Canada
[d] Vaccinology and Immunotherapeutic Program, School of Public Health, University of Saskatchewan, Saskatoon S7N 5E3, SK, Canada
[e] Vaccine and Infectious Disease Organization, 120 Veterinary Road, University of Saskatchewan, Saskatoon S7N 5E3, SK, Canada

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 9 July 2016
Accepted 2 August 2016

Keywords:
VP2 hyper-variable domain
Broiler chickens
Bursal atrophy
Hyper-immunization
Immunosuppression
Maternal antibodies

ABSTRACT

Antibiotic-free and safe animal products are most desirable among consumers. However, ensuring safe poultry products is a challenging task when the chicken immune system is compromised. Infectious bursal disease virus (IBDV) causes immunosuppression and predisposes chickens to secondary infections. Breeder vaccination against IBDV is routinely practiced for producing chicks with maternally-derived antibody (MAb) to prevent infection in newly hatched chicks. The majority of IBDV circulating in Canadian farms are variant strains (vIBDV). Whether circulating vIBDV strains are immunosuppressive in chicks or are amenable to current vaccine regimens has not previously been tested through challenge studies. In this study, one-day-old broiler chicks ($n = 240$) carrying MAb were obtained from broiler breeders vaccinated with commercial IBDV vaccines. In the first set of experiments ($n = 40$/group), at six days post-hatch, one group was challenged with a Canadian field isolate, vIBDV (strain-SK09) (3×10^3 EID_{50}). The second and the third groups (controls) were inoculated with non-immunosuppressive IBDV D-78 (10×10^3 $TCID_{50}$) and saline, respectively. Histopathological examination on days 14 and 30 post-challenge revealed that despite the high level of MAb, vIBDV (SK09) caused severe bursal damage in chicks. Another set of experiments with treatment groups as above, demonstrated that pre-exposure of chicks with vIBDV (SK09) caused immunosuppression resulting in significantly higher mortality and disease severity in chicks challenged with a virulent strain of *Escherichia coli* (*E. coli*). Our data provide evidence that IBDV strains circulating in Canada are immunosuppressive, not amenable to current anti-IBDV vaccination strategy, and a potential threat to antibiotic-free chicken farming.

1. Introduction

Environmental and food safety issues have emerged as a major public health concerns worldwide, as animal products may be contaminated with harmful bacteria (Barrow et al., 1988; Gunawardana et al., 2015; Obeng et al., 2012). Over several decades, antibiotics have been used as feed additives to mitigate early chick mortality due to bacterial infection in chickens, as well as to ensure bacteria-free and safe products to consumers (Diarra and Malouin, 2014; Gaucher et al., 2015). However, there is growing concern about indiscriminate use of antibiotics in animal production and emergence of antibiotic-resistant strains of bacteria that may eventually adversely affect animal and human health (Diarra and Malouin, 2014; Gaucher et al., 2015; Laxminarayan et al., 2013). There have been several studies about raising antibiotic-free chickens, but all of these studies clearly demonstrated that removal of these drugs leads to poor production performance and increases the risk of poultry products being contaminated with food-borne illness-causing bacteria (Engster et al., 2002; Jacob et al., 2008). In May 2014, Chicken Farmers of Canada voluntarily withdrew use of category 1 antibiotics. However, a recent study conducted in Canadian commercial farms reported that drug-free chicken production leads to poor growth performance and greater incidence of *Clostridium perfringens* and *Campylobacter jejuni* infection in chickens (Gaucher et al., 2015). These

Abbreviations: BBW, bursal weight to body weight ratio; CCS, cumulative clinical score; *E. coli*, *Escherichia coli*; EID_{50}, embryo infective dose 50; $TCID_{50}$, tissue culture infective dose 50; GM, geometric mean; H&E, hematoxylin and eosin; IBDV, infectious bursal disease virus; MAb, maternal antibody; SD, standard deviation; SPF, specific pathogen free; cIBDV, classical IBDV; vIBDV, variant IBDV; VP, viral protein.

* Corresponding author at: Department of Veterinary Pathology, Western College of Veterinary Medicine, 52 Campus Dr., University of Saskatchewan, Saskatoon S7N 5B4, SK, Canada.
E-mail address: susantha.gomis@usask.ca (S. Gomis).

http://dx.doi.org/10.1016/j.rvsc.2016.08.002
0034-5288/

studies suggest that implementing antibiotic-free poultry farming could be very challenging (Engster et al., 2002; Gaucher et al., 2015; Jacob et al., 2008), particularly, if chicken's immune system is compromised by immunosuppression, predisposing chickens to several opportunistic pathogens (Amini et al., 2015; Balamurugan and Kataria, 2006; Gaucher et al., 2015). Therefore, preventing immunosuppression becomes increasingly more important in attempts to minimize the use of antibiotics in poultry production.

In chickens, infectious bursal disease (IBD), also called Gumboro disease, is one the most important immunosuppressive diseases, and creates serious problem for the poultry industry worldwide (van den Berg et al., 2000). This disease is caused by IBD virus (IBDV), a highly contagious RNA virus belonging to the family *Birnaviridae* (Balamurugan and Kataria, 2006). IBDV is classified into two distinct serotypes (i.e. serotype I and II). serotype I viruses are pathogenic to chickens and are further classified into classic, variant, and highly virulent strains; whereas serotype II viruses, isolated from turkeys, are apathogenic to chickens (McFerran et al., 1980). IBDV is a non-enveloped virus containing two segments of double stranded RNA (segment A and B) (Mundt et al., 1995). Segment A encodes viral proteins (VPs); VP2, VP3 and VP4; whereas segment B encodes VP1, and VP5 (Van den Berg et al., 1991). VP2 is the major structural protein responsible for binding to neutralizing antibodies (Eterradossi et al., 1997). Within the coding region of VP2 a "hyper-variable domain" exists that contains two major hydrophilic regions (Eterradossi et al., 1997). Substitution mutations in these domains contribute to antigenic drift occurring in the virus, and generate "variant strains" of IBDV (vIBDV).

IBDV in broilers has been controlled by a vaccination strategy to maximize MAb against IBDV by hyper-immunization of broiler breeder parents. A common strategy is to administer a series of live attenuated vaccines followed by an inactivated vaccine "booster" that results in high levels of MAb in the progeny (Tsukamoto et al., 1995). Sometimes, IBDV control measures also combine a broiler vaccination program using attenuated IBDVs (Box, 1988). Significant economic losses in the poultry industry owing to IBDV-induced immunosuppression have been well documented (Fussell, 1998). In the recent past, there has been a significant rise in IBDV infection in Canadian broiler chicken farms associated with production losses, clinical signs associated with respiratory and enteric diseases and vaccine failures (Ojkic et al., 2007). Several studies reported that the majority of the IBDV strains circulating in Canada are variants (Eregae et al., 2013; Ojkic et al., 2007). A recent epidemiological study reported 45 IBDV isolates, 88.89% of the isolates were vIBDV strains, wherein 60% showed high sequence identity to USA isolate vIBDV NC171 and 28.89% were South African 05SA8 strain (Eregae et al., 2013). Besides, a five year epidemiological studies conducted by us on the incidence of IBDV infection in Saskatchewan also revealed that 60%, 20% and 20% of the IBDV isolates were similar to vIBDV strains NC171, DEL-E and 586, respectively (Zachar et al., 2016 (in press). Moreover, recently, we found a strong association of IBDV-induced immunosuppression with the rate of condemnation of broiler carcasses in the broiler chicken industry in Saskatchewan (Amini et al., 2015). Despite several studies in the field, it remained unclear whether circulating vIBDV cause pathogenesis and immunosuppression in chickens; and hence pose a real threat to the poultry industry.

Therefore, the present study investigates the role of circulating vIBDV in causing pathogenesis and immunosuppression in broiler chickens. Challenge experiments were conducted using a Canadian field isolate of IBDV (SK09, which has 98.3% nucleotide sequence identity with NC171), while using non-immunosuppressive IBDV classical strain D78 as a control. The IBDV pathogenesis was studied by measuring the body weight to bursal weight ratio (BBW) and histopathology of the bursa of Fabricius (BF), whereas the immunosuppression of IBDV-infected chickens was evaluated by studying the morbidity and mortality of chickens after an *E. coli* challenge. Results suggest that circulating vIBDV strain SK09 is an immunosuppressive virus and is a potential threat to overall poultry health in Canada.

2. Materials and methods

2.1. Experimental chickens

One-day-old broiler chicks were obtained from a local commercial hatchery (Prairie Pride Chick Sales Ltd., Saskatchewan) in Saskatchewan, Canada. The broiler breeder parent flocks of those broiler chicks had been vaccinated against IBDV at 14 days of age (Bursin 2, Zoetis, Kirkland, Quebec), 21 days of age (Bursimune, Ceva Animal Health, Cambridge, ON), 8 weeks of age (Bursa Blen M, Merial, Gainesville, GA), 10 weeks of age (Matimavac) and 18 weeks of age (Maximune Avi-Pro 432 ND-IB2-BD3 REO, Lohmann Animal Health International, Winslow, ME). Birds were maintained in an isolation facility at the Animal Care Unit (ACU), Western College of Veterinary Medicine (WCVM), University of Saskatchewan. Feed and water were provided ad libitum. This work was approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

2.2. Challenge virus and dose

In this study, vIBDV strain SK09, isolated from broiler chicken farms in Saskatchewan, Canada, was used as the vIBDV challenge virus. This strain has 98.3% nucleotide sequence identity to vIBDV strain NC171 and was selected to represent circulating vIBDV strains, since a recent epidemiological study demonstrated that the majority of circulating strains in Canada have high sequence identity to NC171 (Eregae et al., 2013). These facts make SK09 a good candidate virus to study the pathogenesis and immunosuppression caused by the currently circulating strains in Canadian chicken farms. Furthermore, we selected non-immunosuppressive, classical IBDV strain D78 (Nobilis Gumboro, Intervet International BV) as a control challenge for the direct comparison to assess the impact of SK09-mediated immunosuppression on secondary bacterial infection. Challenge virus (SK09) was prepared following standard procedures (Jackwood et al., 2009). Briefly, pooled bursal tissue samples collected from vIBDV infected birds were homogenized in phosphate buffered saline (PBS) to make 40% (w/v) suspensions. The suspensions were centrifuged 3000 rpm for 10 min and filtered through 45 nm pore size filter. The filtrate was orally administered to 18-day-old specific pathogen free (SPF) chickens (Sunrise Farms, Inc. Catskill, NY). Bursae were collected three days following vIBDV infection and vIBDV was titrated in SPF embryos as described previously (Jackwood et al., 2009). In brief, 10-day-old embryonated SPF chicken eggs were inoculated with 0.1 ml of filtered (45 nm pore size) bursa homogenate diluted 10-fold in PBS solution (pH 7), containing 10 μg/ml gentamicin sulfate (Gibco, Invitrogen Corp.) via the chorio-allontoinc membrane. The embryo infective dose $(EID)_{50}$ was determined using the Reed and Munch method (Jackwood et al., 2009). For the control challenge study, 3×10^3 EID_{50} viral particles of SK09 and 10×10^4 $TCID_{50}$ of IBDV strain D-78 was orally administered per chick.

2.3. Bacteria

An *E. coli* field strain isolated from a turkey with septicemia was used as the challenge strain as described previously (Gunawardana et al., 2015). Briefly, this *E. coli* was nonhemolytic, serum-resistant, serogroup O2; and produced a K1 capsule, aerobactin, and type 1 pili. Aliquots of bacteria in 50% brain heart infusion broth (BHI; Difco, Detroit, MI) supplemented with 25% (w/v) glycerol (VWR Scientific, Inc., Montreal, Quebec, Canada) were stored at −70 °C. For the challenge experiments, bacteria were cultured for 18–24 h at 37 °C on Columbia sheep blood agar plates (Becton, Dickinson and Company, Maryland, USA). A single colony was added into a 250-ml Erlenmeyer flask containing 100 ml