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RESEARCH ARTICLE

Epidemiological Occurrence of the Infectious Bursal Disease Virus in Chicken Flocks Receiving Various Vaccination Regimens

Running Title: Occurrence of Infectious Bursal Disease Virus

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Abstract

Infectious bursal disease (IBD) is an acute immunosuppressive disease of chickens that, despite various vaccination strategies, continues to cause significant economic losses in the poultry industry. The current study sought to determine the prevalence of IBDV infection in relation to various vaccination programs and other risk factors in three different Egyptian Governorates. As a result, 69 chicken flocks were studied clinically and molecularly, with 62 flocks (case flocks) suspected of being naturally infected with the infectious bursal disease virus (IBDV) and 7 flocks (control flocks) apparently healthy. The investigated diseased flocks had whitish diarrhea, depression, ruffled feathers, bursal lesions, nephrosis, nephritis, hemorrhages on muscles, and petechial hemorrhages at the junction of the proventriculus and gizzard. The mortality rate ranged from 0.31 to 25%. Using real-time reverse transcriptase-polymerase chain reaction (RT-PCR), IBDV was detected in 47 of 62 (75.8%) of the tested flocks, while no IBDV was found in the control flocks. The highest prevalence rates were found in chickens aged 18–20 days old and of the Sasso, Indian River, and Hubbard breeds. The detection rate was 100% in chicken flocks vaccinated with Intermediate vaccine (Nobilis Gumboro D78), Intermediate+ Intermediate plus, Vaxxitek-ND-IBD, and Innovax-ND-IBD. Remarkably, the most effective vaccine program was in flocks that used the Vaxxitek-ND –IBD+ Intermediate vaccine (33.3%). It could be concluded that single-dose IBDV vaccines provide insufficient protection against IBDV strains, particularly live ones, due to maternal antibody interference. Meanwhile, vaccination with a recombinant vaccine followed by one or two booster doses of live vaccines provides good protection and prevents IBDV infection. Therefore, it is necessary to utilize the outcome of the field application best trials to update and improve the IBDV immunization programs with keeping in mind the biosecurity practices in chicken farms as well as the complete gene sequencing to detect mutations and virus evolution.

Keywords:
IBD, Epidemiology, Recombinant Vaccines, Real time RT-PCR, Chickens.

Introduction

Infectious bursal disease (IBD) is an acute, highly contagious and immunosuppressive disease of young chickens aged 3–6 weeks [1]. It is characterized by damage of lymphoid tissues, particularly bursa of Fabricius, where B lymphocytes mature and differentiate. It continues to be a major constraint for poultry owners worldwide.

It results in huge economic losses to the poultry industry throughout the world despite extensive vaccination. The socio-economic impacts of IBD are mainly due to i) Direct losses associated with high mortality rates; ii) Indirect losses from immunosuppression, decreased productivity as well as control and prevention expenses [2]. The consequences of immunosuppression are vaccination failure and increased susceptibility of chickens to other pathogens. Furthermore, the infected birds may be good propagators for other viral agents [3].

Chickens, among other domestic poultry, are natural hosts for IBD. The fecal-oral route is the most common route of transmission of the IBDV, after that the aerosol method [4]. IBD can affect both commercial and backyard chickens in an equal way [5]. Additionally, numerous risk factors including location, age, sex, health status, source, and housing system are related to the development of IBD in chickens [6, 7].

Being a member of the family *Birnaviridae* and genus *Avibirnavirus*, infectious bursal disease virus (IBDV), is a bi-segmented, double stranded RNA virus, highly resistant in the environment [8]. The RNA encodes for five viral proteins (VP1 to VP5), with VP2 containing most neutralizing sites and hypervariable regions that allow strains to be classified into multiple antigenic and genetic categories. The IBDV strains are classified as very virulent, virulent, and subclinical depending on the pathogenic type. On the basis of sequencing analysis of the VP2 variable region, the IBDV has been molecularly described. Antigenicity, antibody recognition, immunogenicity, tissue tropism, and pathogenicity of IBDV strains may all be affected by amino acid changes [9, 10].

Since the first discovery of classical IBDV strains in Delaware in 1962 [11], the virus has spread all over the world, while evolving rapidly. Two serotypes (I and II) of IBDV were recognized, but only serotype I causes natural disease in chickens [12]. Through genomic reassortment and recombination events, serotype I variant strains, isolated during the 1980s [13], are able to resist vaccine-induced protection. Furthermore, certain IBDV live vaccines are designed to maintain the quasispecies nature of the virus, which may encourage the development of more virulent antigenic variants or mutants [14].

In Egypt, EL-Sergany *et al.* diagnosed the IBD for the first time based on its specific pathological lesions [15]. IBDV outbreaks continue to infect broiler chickens, resulting in severe economic losses despite mandated vaccination against the disease. Variant and vvIBDV strains have been identified [16- 20].

Live vaccines are categorized as mild, intermediated, intermediate plus, and hot IBD vaccines based on the degree of attenuation [21]. Mild and intermediate vaccines are safer compared with the intermediate plus and hot vaccines because they induce less bursal injury; but are easily neutralized by high levels of maternally derived antibodies (MDA). Next-generation vaccines, which have the advantage of overcoming MDA, have been developed as a result of technological advancements. They are now commercially available in the market such as the IBD vector vaccine which uses turkey herpes virus (HVT) as a vector for the IBDV VP2 gene [22], and the Immune-complex vaccine that is a mixture of the intermediate plus strain with antibodies, which is picked up by macrophages until MDA are no longer present [23]. As a result, effective

vaccines should be used to combat IBDV infections. However, frequent viral mutations, reassortment, and recombination events that can increase virulence of the virus and change its antigenicity might have negative impacts on the vaccination regimes. Moreover, the interference with maternally derived immunity reduces the efficacy of vaccines [24, 25]. Furthermore, due to the emergence of very virulent strains of IBDV, some conventional vaccines have been reported less effective [26].

Although acute IBD is still reported with a significant adverse impact on the poultry industry in Egypt, even in vaccinated chicken flocks, there is a shortage of researches on the epidemiological occurrence of IBD in vaccinated chickens in Egypt. Considering the existing vital situation, we directed the current study to determine the epidemiological occurrence of IBD in both case and control vaccinated chicken farms from three different governorates in Egypt and using different vaccination programs.

Materials and methods

Ethics Declaration

The study was approved by Institutional Animal Care and Use Committee of Zagazig University with approval number ZU-IACUC/2/F/73/2021and was carried out in agreement with the approved guidelines.

Birds and field investigations

The current study was conducted on 69 chicken flocks from three Egyptian governorates: Sharkia, Port Said, and Ismailia, during the period from February 2020 to November 2022. These examined flocks were allocated into two groups; i) apparently healthy chickens with no observable clinical signs (control flocks; n = 7) and ii) suspected to be naturally infected with IBDV, presenting clinical and postmortem findings signifying IBDV infection (case flocks; n = 62). The flocks' histories comprising total number of birds, age, breed, season, rearing system, previous vaccination, clinical sings, mortality rates, and postmortem lesions of freshly dead birds were recorded. Descriptive data about these investigated chicken flocks was illustrated in Tables 1 and 2.All investigated case and control flocks were under the umbrella of various IBDV vaccination regimens. Three birds / flock were collected and submitted to the laboratory of Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Zagazig University for IBD virus detection.

Table 1. Descriptive data and infectious bursal disease (IBD) vaccine regimens history of the investigated chicken Case-flocks during 2020-2022.

Flock No.	Year	Flock density	Breed	Age (days)	Vaccination		Locality	Season	Rearing system
					Type of vaccine	Age (days)			
1	2022	50000	Ross	20	Nobilis Gumboro D78	9	Port said	Winter	Closed
2	2022	48000	Ross	21	Nobilis Gumboro D78	12	Port said	Winter	Closed
3	2022	49000	Ross	31	Nobilis Gumboro D78	11	Port said	Spring	Closed
4	2022	49000	Arbor Acres	29	Nobilis Gumboro D78	11	Port said	Summer	Closed
5	2022	51000	Arbor Acres	25	Nobilis Gumboro D78	11	port said	Autumn	Closed
6	2022	76000	Arbor Acres	21	Vaxxitek-IBD	1	Sharkia	Winter	Closed
7	2022	76000	Arbor Acres	21	Vaxxitek-ND-IBD	1	Sharkia	Spring	Closed
8	2022	75000	Arbor Acres	29	Vaxxitek-IBD Nobilis Gumboro D78	1 11	Sharkia	Summer	Closed
9	2022	75000	Arbor Acres	29	Vaxxitek-ND-IBD Nobilis Gumboro D78	1 11	Sharkia	Summer	Closed
10	2022	74000	Arbor Acres	27	Vaxxitek-ND-IBD Nobilis Gumboro D78	1 11	Sharkia	Autumn	Closed
11	2022	71000	Arbor Acres	29	Innovax-ND-IBD UNIVAX-BD	1 10	Sharkia	Autumn	Closed
12	2022	35000	Balady	24	Vaxxitek-IBD	1	Sharkia	Winter	Opened
13	2022	36000	Balady	24	Vaxxitek-IBD	1	Sharkia	Spring	Opened
14	2022	61000	Indian River	21	Innovax-ND-IBD Nobilis Gumboro D78	1 11	Sharkia	Winter	Closed
15	2022	60000	Indian River	21	Innovax-ND-IBD Nobilis Gumboro D78	1 11	Sharkia	Spring	Closed
16	2022	59000	Ross	29	Transmune IBD complex Bursine-2	1 11	Sharkia	Spring	Closed
17	2022	61000	Ross	29	Transmune IBD complex Bursine-2	1 11	Sharkia	Summer	Closed
18	2022	60000	Ross	27	Transmune IBD complex Bursine-2	1 11	Sharkia	Autumn	Closed
19	2022	111000	Balady	24	Innovax-ND-IBD	1	Ismailia	Winter	Closed

					Bursien-2 Bursine-2	12 22			
20	2022	25000	Balady	22	Innovax-ND-IBD	1	Sharkia	Winter	Opened
21	2022	33000	Balady	24	Innovax-ND-IBD	1	Sharkia	Spring	Opened
22	2022	30000	Balady	28	Vaxxitek-ND-IBD Nobilis Gumboro D78	1 12	Sharkia	Summer	Opened
23	2022	166000	Cobb	19	Innovax-ND-IBD	1	Port said	Winter	Closed
24	2022	160000	Ross	23	Innovax-ND-IBD AviPro IBD Xtreme	1 12	Port said	Spring	Closed
25	2022	160000	Ross	35	Innovax-ND-IBD AviPro IBD Xtreme	1 12	Port said	Spring	Closed
26	2022	150000	Cobb	19	Vaxxitek-ND-IBD	1	Ismailia	Winter	Closed
27	2022	150000	Ross	23	Vaxxitek-ND-IBD AviPro IBD Xtreme	1 12	Ismailia	Spring	Closed
28	2022	150000	Ross	35	Vaxxitek-ND-IBD AviPro IBD Xtreme	1 12	Ismailia	Spring	Closed
29	2022	22000	Ross	23	BURSIMUNE IBD BLEN (2512)	10 14	Sharkia	Winter	Opened
30	2022	23000	Ross	19	BURSIMUNE IBD BLEN (2512)	9 13	Sharkia	Spring	Opened
31	2022	20000	Ross	27	Nobilis Gumboro D78 Bursine- plus	11 15	Sharkia	Spring	Opened
32	2022	23000	Ross	33	Vaxxitek-IBD Nobilis Gumboro D78	1 11	Sharkia	Summer	Opened
33	2022	22000	Cobb	35	Vaxxitek-IBD Nobilis Gumboro D78	1 11	Sharkia	Autumn	Opened
34	2022	28000	Balady	24	Vaxxitek-IBD	1	Sharkia	Winter	Opened
35	2022	25000	Balady	26	Transmune IBD complex Bursine-2	1 11	Sharkia	Spring	Opened
36	2022	25000	Balady	26	Vaxxitek-ND-IBD Bursine-2	1 12	Sharkia	Summer	Closed
37	2020	55	Balady	60	NA	-	Sharkia	Autumn	Opened
38	2020	500	Cobb	29	NA	-	Sharkia	Winter	Opened
39	2020	51	Balady	50	NA	-	Sharkia	Spring	Opened

40	2020	40	Balady	45	NA	-	Sharkia	Spring	Opened
41	2020	90	Balady	75	NA	-	Sharkia	Summer	Opened
42	2020	25	Balady	60	NA	-	Sharkia	Winter	Opened
43	2020	80	Balady	60	NA	-	Sharkia	Autumn	Opened
44	2020	40	Balady	90	NA	-	Sharkia	Autumn	Opened
45	2020	40	Sasso	40	NA	–	Sharkia	Winter	Opened
46	2020	700	Ross	30	CEVAC IBDL	14	Sharkia	Winter	Opened
47	2020	64	Cobb	20	NA	-	Sharkia	Winter	Opened
48	2020	20	Sasso	40	NA	–	Sharkia	Spring	Opened
49	2020	50	Balady	30	NA	-	Sharkia	Spring	Opened
50	2020	10000	Balady	36	Vaxxitek-IBD Intermediate vaccine	1 10	Sharkia	Spring	Closed
51	2020	50	Balady	30	NA	–	Sharkia	Spring	Opened
52	2021	10000	Balady	36	Vaxxitek-IBD Intermediate	1 10	Sharkia	Winter	Closed
53	2021	50	Balady	30	NA	–	Sharkia	Winter	Opened
54	2021	50	Cobb	21	NA	–	Sharkia	Winter	Opened
55	2021	6000	Balady	27	CEVAC IBDL	13	Sharkia	Winter	Opened
56	2021	12000	Cobb	30	Vaxxitek-IBD	1	Sharkia	Summer	Closed
57	2021	4000	Ross	28	Nobilis Gumboro D78 Nobilis Gumboro 228E	11 14	Sharkia	Spring	Opened
58	2021	5500	Balady	18	AviPro IBD Xtreme	12	Sharkia	Summer	Opened
59	2021	60000	Indian River	33	Innovax-ND-IBD Nobilis Gumboro D78	1 10	Sharkia	Summer	Closed
60	2021	11000	Balady	40	Vaxxitek-IBD Nobilis Gumboro D78	1 10	Sharkia	Summer	Closed
61	2021	3200	Hubbard	25	CEVAC IBDL CEVAC IBDL	10 14	Sharkia	Spring	Opened
62	2022	4000	Ross	26	CEVAC IBDL	12	Sharkia	Winter	Opened

NA: not available; Nobilis Gumboro D78, Bursine2 &-plus, BURSIMUNE and UNIVAX-BD are intermediate; Vaxxitek-IBD, Vaxxitek-ND-IBD, Innovax ND-IBD and are recombinant, AviPro IBD Xtrem , CEVAC IBDL and IBD BLEN (2512) are intermediate plus and Transmune IBD complex is immunocomplex vaccines.

Table 2: Descriptive data and infectious bursal disease (IBD) vaccine regimens history of the investigated chicken control-flocks during 2022.

Flock No.	Year	Flock density	Breed	Age (day)	Vaccination		Locality	Season	Rearing system
					Type of vaccine	Age (days)			
1	2022	30000	Balady	28	Vaxxitek-IBD Bursine-2	1 12	Sharkia	Summer	Opened
2	2022	111000	Balady	28	Vaxxitek-ND-IBD AviPro IBD Xtreme AviPro IBD Xtreme	1 11 20	Ismailia	Spring	Closed
3	2022	111000	Balady	28	Vaxxitek-IBD Bursine-2 Bursine-2	1 12 17	Ismailia	Summer	Closed
4	2022	160000	Ross	28	Vaxxitek-ND-IBD Bursine-2	1 12	Port said	Summer	Closed
5	2022	158000	Ross	28	Innovax-ND-IBD Bursine-2	1 12	Port said	Autumn	Closed
6	2022	150000	Ross	28	Vaxxitek-ND-IBD Bursine-2	1 12	Ismailia	Summer	Closed
7	2022	147000	Ross	28	Vaxxitek-ND-IBD Bursine-2	1 12	Ismailia	Autumn	Closed

Nobilis Gumboro D78, Bursine-2, BURSIMUNE and UNIVAX-BD are intermediate,Vaxxitek-IBD, Innovax-ND-IBD and Vaxxitek-ND-IBD are recombinant, AviPro IBD Xtrem , CEVAC IBDL and IBD BLEN (2512) are intermediate plus and Transmune IBD complex is immunocomplex vaccines.

Sample collection

Sixty-nine pooled bursae of Fabricius samples (3 bursae / flock/ pool) were collected from 69 vaccinated chicken flocks, located in three Egyptian governorates, under complete aseptic conditions and kept at -20^{o}C till be used in IBDV detection using real-time reverse transcription polymerase chain reaction (real-time RT-PCR).

RNA extraction

The viral RNAs were extracted from 69 pooled bursal homogenates, one part of each pooled bursa sample mixing in sterile saline (1:1), representing 62 vaccinated chicken case flocks and 7 vaccinated chicken control flocks using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Real time RT-PCR

The extracted RNA was exposed to one-step real time RT-PCR by using QuantiTect Probe RT-PCR Kit (Qiagen) for detection of IBDV using the probe and primer pair targeting the VP2 gene of IBDV. A reference IBDV strain and non-infected bursa were used as positive and negative controls, respectively. The IBDV/SHEM-8/2015 with accession no. MK493463 was used as a positive control and was obtained from Dr. Tamer A. El-Aried, Reference Laboratory for Quality control on Poultry Production, Sharkia Branch, Zagazig, Egypt. The probe and primers sequences were: IBDV probe: 5'-(FAM) TCCCCTGAAGATTGCAGGAGCATTT G-(TAMRA)-3'; IBDV forward primer: 5'-GAGGTGGCCGACCTCAACT-3'and IBDV reverse primer: 5'-AGCCCGGATTATGTCTTTGAAG-3' [27]. The thermal cycling conditions were 45°C for 10 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec, 57°C for 30 sec and 72°C for 30 sec.

Statistical Analysis

GraphPad Prism version 8 for Windows, GraphPad Software, La Jolla, California, USA, www.graphpad.com, was used to determine the effect of numerous risk factors on the prevalence of IBD in vaccinated chicken flocks. The results with $P < 0.01$ were considered as statistically significant.

Results

Clinical and postmortem findings

In apparently healthy chicken flocks (control flocks), no noticeable clinical signs or postmortem lesions of clinical disease were observed. Meanwhile, flocks suspected to be naturally infected with IBDV (case flocks) showed clinical signs in the form of whitish diarrhea (29/62; 46.8%) (Figures 1A and B), ruffled feathers, depression (12/62; 19.4%) (Figure 1C) and decrease in growth rate (19/62; 30.6%). Moreover, greenish diarrhea (7/62; 11.3%), congested head (8/62; 13%) and respiratory signs (25/62; 40.3%) were observed. The mortality rates of the examined flocks ranged from 0.31–25% (Table 3). The mortality percentages of the investigated chickens vaccinated with different IBDV vaccines were demonstrated in Table 4. Interestingly, the lower mortality rates were recorded in chicken flocks vaccinated with Transmune IBD complex + Intermediate (1.16 %), Vaxxitek-IBD + Intermediate (1.52%) and Intermediate plus (1.97%).

Table 3. Clinical signs, postmortem lesions and mortality rates of chickens from infectious bursal disease virus (IBDV) positive flocks during 2020 to 2022

Flock No.	Mortality rate (%)	Clinical signs					PM lesions					
		Respiratory signs	Congested head	Diarrhea	Depression/ ruffled feather	Decrease growth rate	Bursal lesions	Hemorrhages on muscles	Hemorrhages at junction between proventriculus and gizzard	Kidney lesions	Caseated plug in tracheal bifurcation	Fibrinous pericarditis perihepatitis air sacculitis
1	4.66 (21d to 28d)	+	-	-	-	+	Enlarged	-	-	Nephrosis	+	+
2	5.11 (21d to 28d)	+	-	-	-	+	Enlarged	-	-	Nephritis	+	+
3	5.06 (28d to 37d)	-	-	Greenish	-	+	Enlarged	-	-	Nephrosis	-	-
4	1.42 (28d to 35d)	-	-	-	-	-	Enlarged	-	-	Nephritis	-	-
5	1.33 (21d to 28d)	+	-	-	-	+	Enlarged	-	-	Nephritis	-	+
6	4.26 (21d to 28d)	-	+	-	-	+	Atrophy	-	-	Nephrosis	+	+
7	3.23 (21d to 28d)	+	-	Greenish	-	+	Atrophy	-	+	Nephritis	-	+
8	2.99 (27d to 35d)	+	-	-	-	-	Enlarged	-	-	Nephritis	-	+
9	0.39 (21d to 28d)	-	-	-	+	-	Atrophy	-	-	-	-	-
10	5.09 (21d to 28d)	+	+	-	-	+	Atrophy	-	-	Nephrosis	+	+

11	3.105 (21d to 28d)	+	+	Whitish greenish	-	+	Enlarged	Thigh	-	Nephrosis	+	-
12	1.34 (28d to 35d)	+	-	Whitish	-	-	Enlarged	-	-	Nephritis	-	+
13	0.72 (21d to 28d)	-	-	-	+	-	Enlarged	-	-	-	-	-
14	1.052 (21d to 28d)	-	-	-	+	-	Enlarged	-	-	-	-	-
15	0.93 (21d to 28d)	-	-	-	+	-	Enlarged	-	-	Nephritis	-	-
16	14.72 (19d to 28d)	+	-	-	-	+	Enlarged	-	-	Nephrosis	+	+
17	14.18 (21d to 28d)	+	+	Greenish	-	+	Atrophy	-	-	Nephrosis	+	+
18	5.31 (33 d to 39d)	+	+	Whitish	-	+	Atrophy	-	-	Nephrosis	-	-
19	13.77% (19d to 28d)	+	-	-	-	+	Enlarged	-	-	Nephrosis	+	+
20	21.6 (21d to 29d)	+	+	Greenish	-	+	Atrophy	-	-	Nephrosis	+	+
21	7.46 (21d to 28d)	+	-	-	-	+	Atrophy	-	-	Nephrosis	+	+
22	6.38 (19d to 28d)	+	+	Greenish	-	+	Atrophy	-	-	_	-	+
23	4.61 (27 to 35 d)	+	-	Greenish	-	_	Enlarged	-	-	Nephritis	-	+
24	2.1 (28 to 35 d)	-	-	-	-	_	Enlarged	-	-	Nephritis	-	-

25	4.14 (21d to 28d)	-	-	-	+	-	Enlarged	Thigh	-	Nephrosis	-	-
26	2.97 (21d to 28d)	+	-	-	-	-	Enlarged	Thigh	-	Nephrosis	-	+
27	18.18	+	-	Whitish	-	-	Enlarged	-	+	-	-	+
28	3.92	-	-	Whitish	-	-	Enlarged	Thigh	-	Nephrosis	-	-
29	10	+	-	Whitish	-	-	Enlarged	-	+	Nephrosis	-	+
30	12.22	+	-	Whitish	-	-	Enlarged	Thigh and breast	-	Nephrosis	-	+
31	20	+	+	Whitish	-	-	Enlarged	-	-	Nephrosis	-	+
32	17.5	-	-	Whitish	+	-	Enlarged	Thigh	+	Nephrosis	-	-
33	25	-	-	Whitish	+	-	Enlarged	Thigh	+	Nephrosis	-	-
34	6.25	-	-	Whitish	-	-	Enlarged	-	-	Nephrosis	-	-
35	-	-	-	Whitish	-	-	Enlarged	Thigh	+	Nephrosis	-	-
36	-	-	-	Whitish	-	-	Enlarged	Thigh	-	Nephrosis	-	-
37	0	-	-	Whitish	-	-	Enlarged	Thigh and breast	+	Nephrosis	-	-
38	6	+	-	Whitish	-	-	Enlarged	Thigh and breast	-	Nephrosis	-	+
39	18	+	-	-	+	+	-	Thigh	+	Nephrosis	-	+
40	1.65	-	-	Whitish	-	-	Enlarged	-	-	Nephrosis	-	-
41	0.53	-	-	Whitish	-	+	Enlarged	-	-	Nephrosis	-	-
42	2.025	-	-	Whitish	-	-	Enlarged	-	-	Nephrosis	-	-
43	1.38	-	-	Whitish	+	-	Enlarged	-	-	Nephrosis	-	-
44	0.31	-	-	Whitish	-	-	Enlarged	-	-	Nephrosis	-	-
45	0.95	-	-	Whitish	-	+	Enlarged	-	-	Nephrosis	-	-
46	2.06	+	-	Whitish	-	-	Enlarged	-	-	Nephrosis	-	+
47	3.55	+	-	Whitish	-	-	Enlarged and hemorrhagic	Thigh	-	Nephrosis/ nephritis	-	+

+ mean present, - mean absent, PM: postmortem

Table 4: Mortality percentages of investigated chickens vaccinated with different infectious bursal disease virus vaccines

Type of vaccine	No. of examined birds	No. of dead birds	Mortality %
Intermediate (Nobilis Gumboro D78)	247000	8645	3.5
Intermediate plus	19400	383	1.97
Intermediate + Intermediate plus	69000	4227	6.13
Vaxxitek-IBD	187000	5130	2.74
Vaxxitek-IBD + Intermediate	151000	2293	1.52
Vaxxitek-ND-IBD	226000	23116	10.23
Vaxxitek-ND-IBD + Intermediate	504000	42970	8.53
Innovax-ND-IBD	224000	24433	11
Innovax-ND-IBD + Intermediate	683000	38261	5.6
Transmune IBD complex + Intermediate	205000	2385	1.16

Postmortem lesions of the naturally infected IBD flocks were noticed in bursae (Figures 1D-F) in the form of enlarged (49/62; 79%), enlarged with petechial hemorrhage in the mucosa (1/62; 1.6 %) or gelatinous exudates (1/62; 1.6%) and atrophied (12/62; 19.4%) in some investigated chickens. Nephrosis (40/62; 64.5%), nephritis (16/62; 25.8%) and nephrosis & nephritis (1/62; 1.6%) with extension of ureters with urates were recorded in the examined chickens (Figures 1G and H). Additionally, hemorrhages on the thigh (Figure 1I) and pectoral muscles were noticed (13/62; 21%) and petechial hemorrhages at the junction between the proventriculus and gizzard (8/62; 13%) were observed. Nevertheless, some investigated birds showed respiratory manifestations, the gross examination exposed septicemia, fibrinous pericarditis, perihepatitis and airsacculitis (24/62; 38.7%) and caseated plugs in tracheal bifurcation (10/62; 16.1%). Moreover, just one studied flock (Flock no. 7) showed hemorrhages on the cecal tonsils, and two flocks (Flocks no. 11 and 32) showed friable livers.

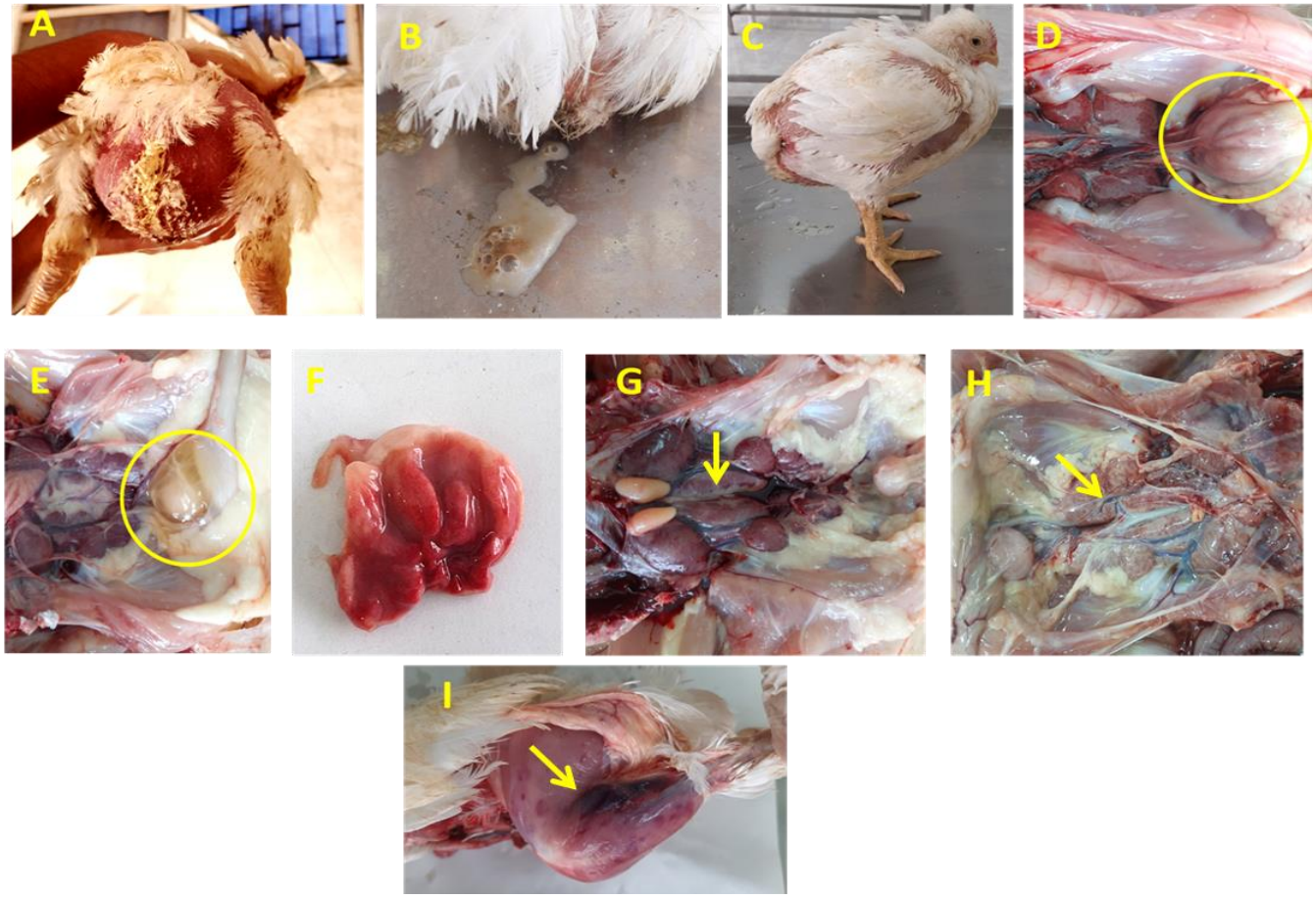
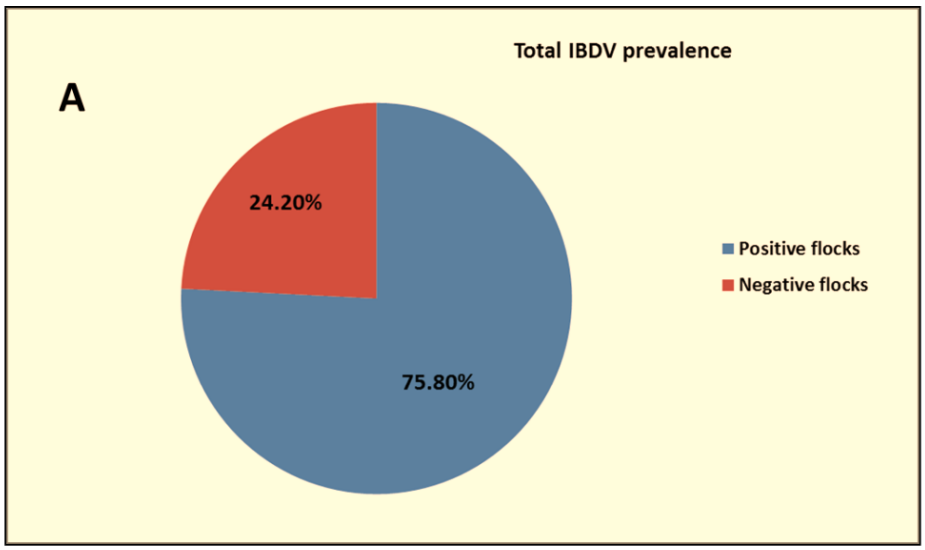


Figure 1. Clinical and postmortem findings of chickens suspected to be affected by IBDV. (A) Whitish diarrhea soiled vent feathers. (B) Profuse white yellowish watery diarrhea. (C) Depression and ruffled feathers. (D) Closed enlarged bursa. (E) Closed bursa filled with gelatinous exudate. (F) Opened hemorrhagic bursa. (G) Kidney showing nephritis. (H) Kidney showing nephrosis. (I) Hemorrhages on thigh muscle.

Prevalence of IBDV in the investigated chicken flocks

All bursal samples collected from apparently healthy chickens were negative for IBDV using real-time RT-PCR. Meanwhile, in IBD-suspected flocks, IBDV was detected in the collected bursae of Fabricius from three governorates in Egypt with an overall prevalence of 47/62; 75.8% but none of the apparently healthy flocks revealed IBDV (0/7) (Figure 2A and Table 3).



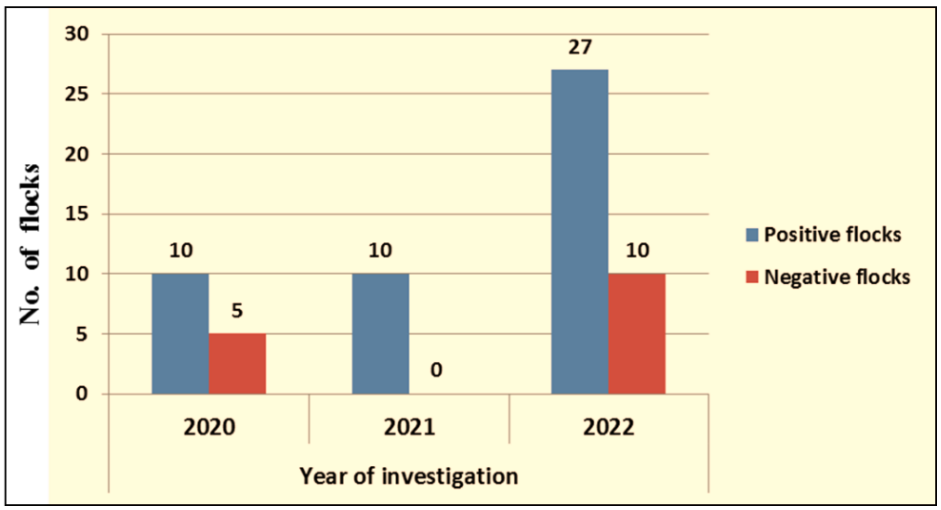


Figure 2. The occurrence of IBDV among IBD-suspected chicken flocks in three governorates in Egypt. (A): Total IBDV prevalence. (B): Detection of IBDV in the chicken flocks during the studied period of investigation.

The occurrence rate of IBDV in the examined chickens was documented according to several potential risk factors; flock density, breed, age, season, locality, rearing system, years of investigation, and type of vaccines. The molecular detection of IBDV in the chicken flocks during the studied period of investigation presented that the IBD prevalence was higher during 2021(10/10; 100%) as compared to 2022 and 2020 (27/37; 72.9% and10/15; 66.6%) (Figure 2B). The IBDV detection was significantly (P < 0.01) higher among chicken farms with flock density more than 100000 chickens (85.7%).In addition, the infection rates were higher in chickens aged 18-20 days (100%) and >35 days (91.7%).The chicken Sasso, Indian River and Hubbard were the most affected breeds (100%) as compared to Ross (76.5%), Balady (75%), Cobb (71.4%) and Arbor Acres (62.5%) ones. The prevalence of IBD was significantly (P< 0.01) higher among chickens in Port Said (100%) as compared to Ismailia (75%) and Sharkia governorates (72%) (Table 5).

Table 5. Risk factors associated with prevalence of infectious bursal disease (IBD) in the surveyed chicken flocks during 2020-2022

Risk factor	No. of examined flocks	No. of positive flocks (%)	Mean Diff.	95% CI of diff.	*P* value
Flock density					
≤ 5000	19	14 (73.7)	-4.5	-10.96 - 1.959	0.1238
>5000 - ≤ 50000	23	19 (82.6)	RF	-	-
>50000 -≤ 100000	13	8 (61.5)	10.5	4.041-16.96	0.013*
>100000	7	6 (85.7)	14.5	8.041- 20.96	0.0051**
Breed					
Ross	17	13 (76.5)	-6	-12.90 - 0.8979	0.0884
Arbor Acres	8	5 (62.5)	-14.5	-21.40 - -7.602	0.0012**
Balady	24	18 (75)	RF	-	-
Indian River	3	3 (100)	18	11.10- 24.90	0.0004***
Cobb	7	5(71.4)	15	8.102 -21.90	0.001**
Sasso	2	2(100)	19	12.10 - 25.90	0.0003***
Hubbard	1	1(100)	20	13.10 - 26.90	0.0002***
Age(d)					
18-20	6	6 (100)	RF	-	-
21-28	26	22 (84.6)	-18	-33.36 - -2.645	0.0326*
28-35	18	8 (44.4)	-7	-22.36 - 8.355	0.3002
>35	12	11 (91.7)	-5.5	-20.86 - 9.855	0.4422
Locality					
Sharkia	50	(72)36	RF	-	-
Ismailia	4	3 (75)	39.5	6.967 -72.03	0.0345*
Port said	8	8 (100)	35	2.467 -67.53	0.0435*
Season					
Summer	12	7 (58.3)	RF	-	-
Spring	21	17 (81)	-9.5	-13.79 to -5.206	0.0053**
Autumn	8	4(50)	3.5	-0.7937 - 7.794	0.0846
Winter	21	19 (90.5)	-10.5	-14.79 to -6.206	0.004**
Rearing system					
Opened	33	25 (75.8)	RF	-	-
Closed	29	22 (75.9)	3.5	-9.065 to 16.06	0.1725

RF = reference factor, $P < 0.01$ was considered as statistically significant

Also, the occurrence of IBD was significantly ($P < 0.01$) higher among chickens during winter and spring seasons with the percentages of 90.5% and 81%, respectively. According to rearing system there was no significant difference ($P > 0.01$) in the incidence of IBD between opened (75.8%) and closed (75.9%) systems (Table 5). Regarding different IBD vaccination programs, the prevalence of IBD was 100% in poultry flocks that used Intermediate vaccine (Nobilis Gumboro D78) only, Intermediate+ Intermediate plus, Vaxxitek-ND-IBD and Innovax-ND-IBD. But flocks received Vaxxitek-ND-IBD + Intermediate vaccines revealed (33.3%), Transmune IBD complex + Intermediate vaccine (50%), and Vaxxitek-IBD +Intermediate vaccine (66.7%) (Table 6).

Table 6. Prevalence of infectious bursal disease virus (IBDV) infection among chicken flocks with different vaccination regimens

Risk factor	No. of examined flocks	No. of positive flocks (%)	Mean Diff.	95% CI of diff.	*P* value
Type of vaccine					
Intermediate (Nobilis Gumboro D78)	5	5 (100)	3	-0.6917 to 6.692	0.1381
Intermediate plus	5	4 (80)	2.5	-1.192 - 6.192	0.2787
Intermediate+ Intermediate plus	4	4 (100)	2	-1.692 - 5.692	0.5145
Vaxxitek-IBD	5	4 (80)	2.5	-1.192 - 6.192	0.2787
Vaxxitek-IBD +Intermediate	6	4 (66.7)	3	-0.6917 - 6.692	0.1381
Vaxxitek-ND-IBD	2	2(100)	RF	-	-
Vaxxitek-ND-IBD + Intermediate	6	2 (33.3)	-2	-5.692 - 1.692	0.5145
Innovax-ND-IBD	3	3 (100)	-1	-4.692 - 2.692	0.9724
Innovax-ND-IBD + Intermediate	7	6 (85.7)	-4.5	-8.192 --0.8083	0.0156*
Transmune IBD complex + Intermediate	4	2 (50)	-1	-4.692 - 2.692	0.9724
NA	15	11(73.3)	-	-	-
Total	62	47	-	-	-

RF = reference factor; *P*< 0.01 was considered as statistically significant; NA: not available.

Discussion

Infectious bursal disease is the most important contagious immunosuppressive disease of poultry [28] and increasing the susceptibility to many infectious agents that are non-pathogenic in healthy chickens [3]. The control of IBDV infection depends mainly on vaccination, but recently, IBDV field strains partially fled form vaccines due to mutation and reassortment or recombination that increase viral pathogenicity and virulence [24, 25]. In Egypt, IBD outbreaks have still occurred even in vaccinated chicken flocks leading to serious economic losses to the poultry industry [17, 18, 29, 30]. Therefore, the aim of the current study was to investigate molecularly the prevalence of IBDV in chicken farms using different vaccination programs allocated in three different provinces in Egypt during the period from 2020 to 2022.

Clinically, the diagnosis of IBD depends on the observation of symptoms and postmortem examination of the bursa of Fabricius [16, 31]. In the current study, the investigated chickens exhibited clinical signs comprising depression, ruffled feathers and whitish diarrhea and 0.31–25% mortality rate. The gross lesions were enlarged, hemorrhagic, and atrophied bursa, bursa filled with gelatinous exudate, hemorrhages on the thigh and pectoral muscle, and petechial hemorrhages at the junction between the proventriculus and gizzard. Swelling of the kidneys and ureters extended with urates were also observed. The aforementioned clinical picture was previously presented to be accompanied with IBDV infection by several authors [32- 35].

We clarified that the concurrent infections with other viruses and bacteria (Newcastle disease virus (NDV), IBV, and *Escherichiacoli*) might play a role in complicating the clinical picture of IBDV-infected birds. Such coexisting infections were manifested in the present study through allying of additional clinical signs and gross lesions including; respiratory signs, a congested head, greenish diarrhea, septicemia, fibrinous pericarditis, perihepatitis and airsacculitis, hemorrhages on the cecal tonsils and caseated plugs in tracheal bifurcation. IBDV mixed viral and /or bacterial infections are common and comparable findings have been reported previously [33, 36].

Nevertheless, all the surveyed chicken flocks were vaccinated, this study recorded mortality rates ranged from 0.31–25%, which was consistent with another previous study, carried out in Egypt, where the mortality were from 2–20% [33]. On the other hand, Omer & Khalafalla and Al-khalefa *et al*. documented higher mortality rates with the percentages of 76% and 40%, respectively [20, 37]. This could be attributed to the differences in the vaccination programs and presence or absence of concurrent infections.

In this study, the real-time RT-PCR results confirmed the presence of IBDV in 47/62 (75.8%) chicken flocks and none of the apparently healthy flocks revealed IBDV. This result revealed that not all clinically diagnosed IBD flocks were positive. Interestingly, all these positive flocks were vaccinated against IBDV, indicating IBDV outbreaks in the vaccinated flocks, as previously documented [18, 30, 38, 39].Differently, higher IBDV prevalence rates were documented in vaccinated chickens in Egypt (17/20; 85%) [20] and Khartoum State, Sudan;100% [37]. Meanwhile, lower percentages of IBDV infection

were previously recorded in several studies; 24.07% [40], 30% [41], 56.25 % [42] and 57.1% [33].

Notably, there are various risk factors, including flock density, season, age, breed housing system, region of investigation, and vaccination regimens, associated with the occurrence of IBD in chickens. In the present study, the high stocking density increased the detection of IBDV as recorded before [33]. The common ages of the studied IBD flocks in most of the previous researches were ranged from 3–6 weeks [43, 44]. Likewise, our results exhibited that 91.7% (11/12) of the investigated chicken flocks were from flocks aged >35 days. Similar reports have been described previously [45, 46], where these authors reported that the susceptibility of chickens to IBDV is influenced by their ages, reaching its peak at 4 weeks of age. Additionally, this is also consistent with an earlier study carried out in Khartoum State, Sudan, demonstrating that 70% of IBD outbreaks occurred in vaccinated chickens at the age of 6 and 8 weeks [37]. Remarkably, in this study, IBDV was identified in 100% (6/6) of the IBDV-suspected chickens aged 18–20 days. This result was close to those listed earlier in another previous study conducted in Egypt where IBDV infection was detected in 60.7% (17/28) of the affected birds below 3 weeks of age [33].

The occurrence of IBD was higher between commercial broiler chickens such as Sasso, Indian River, and Hubbard chickens (100%) than in local chicken breeds, indicating that the local chicken breeds were more resistant to the infection [47, 48]. Accordingly, it is noteworthy that 3 out of the 7 apparently healthy flocks presented in this study were balady flocks. In addition, seasonal variation affects the incidence of IBD where, in the current study, the IBD prevalence rates were higher among chickens collected during the winter (19/21; 90.5%) and spring (17/21; 80.9%) seasons, and this is not similar to previous studies that reported that the incidence increased in the summer season[33, 35, 47]. This can be attributed to the fact that the majority of the investigated flocks in this study were collected during the winter and spring seasons. According to the rearing system, there was no significant difference in incidence of IBD between opened (75.8%) and closed systems (75.9%). This is not similar to previous studies, which reported that the IBD incidence increased in chickens housed in opened system due to frequent exposure to immunosuppressive factors such as heat stress, deprivation of water, and poor nutrition which resulted in suppression of the chicken immune system [49, 50].

Notably, there are many vaccination programs for preventing IBDV infection in chickens that differ in vaccine (s) type, vaccination age, route of vaccine administration, vaccine frequency, vaccine handling and transportation, and interference with MDA. The half-life of the MDA and their homogeneity or heterogeneity is essential to deciding the optimal time of vaccination [51]. The currently used vaccines in many countries, including Egypt, are imported and might not be antigenically similar to the currently circulated field strains; accordingly, IBD outbreaks still occur in the vaccinated flocks [39, 52]. In the present investigation, it was noted that the incidence of IBDV was noted in diseased flocks applied different vaccination programs with an incidence rate of 100% in chicken flocks that used the intermediate vaccine (Nobilis Gumboro D78) only, Intermediate+ Intermediate plus, Vaxxitek-ND-IBD, and Innovax-

ND-IBD. This result indicates vaccination failure that may be attributed to various causes, as the high MDA at the time of IBDV vaccination might interfere with the vaccine response [53, 54], improper vaccination timing, faulty vaccine application, mishandling of vaccines especially recombinant ones, and vaccine strains that might not be antigenically similar to the currently circulating Egyptian field strains [39, 52]. These results highlight the urgent need for partial or complete sequencing of both vaccine and currently circulating field virus genomes. Additionally, the antigenic matching between the vaccine(s) and epidemic circulating strains is correspondingly very critical.

Interestingly, from the current results, the most effective vaccine program was in farms that used the Vaxxitek-ND-IBD + Intermediate vaccine, which gave good protection. The lower mortality rates were recorded in chicken flocks vaccinated and succeeded to prevent IBDV infection. This can be attributed to the NDV F gene insertion site is the same as the IBD VP2 gene, and the use of a single promotor allows reliable antigen expression. The turkey herpes virus double construct vaccine (HVT-ND-IBD) was tested in the field in birds with MDA. Efficacy against Marek's disease, Newcastle disease, and IBD was evaluated after both *in-ovo* vaccination in broilers and subcutaneous vaccination in commercial layer birds and it was confirmed that HVT-ND-IBD was able to provide protection against all these diseases [55]. Interestingly, the efficacy of recombinant IBDV vaccines boostered with one or two booster dose (s) of live vaccines is lacking and needs several investigations.

Conclusion

Regardless of different vaccination programs, IBDV still circulates among chickens in Sharkia, Port Said, and Ismailia governorates, Egypt. The current IBDV vaccines applied in one dose provide inadequate protection against IBDV strains. Meanwhile, vaccination with a recombinant vaccine followed by one or two booster dose (s) of live vaccines, giving good protection and preventing IBDV infection in apparently healthy flocks. Therefore, the currently applied IBDV vaccination strategies should be revised and improved as well as comprehensive genotyping identification for both VP1 and VP2 to detect any virus evolution.

Conflict of Interest

There is no conflict of interest to declare.

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الملخص العربي

التواجد الوبائي لفيروس مرض التهاب جراب فابريشيا المعدي في قطعان الدجاج المحصنة ببرامج مختلفة

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مرض إلتهاب جراب فابريشياالمعدي (IBD) هو مرض مثبط حاد للمناعة يصيب الدجاج ولا يزال يسبب خسائر إقتصادية فادحة لصناعة الدواجن على الرغم من تطبيق برامج تحصين مختلفة. و تهدف الدراسة الحالية إلى تقصى مدى إنتشار عدوى فيروس مرض إلتهاب جراب فابريشيا المعدي فيما يتعلق ببرامج التحصين المختلفة وعوامل الخطر الأخرى في ثلاث محافظات مختلفة بمصر. لذلك تم فحص عدد 69 من قطعان الدجاج؛62 منهم يشتبه في إصابتها طبيعيًا بفيروس مرض التهاب جراب فابريشيا المعدي (IBDV) و7 قطعان تبدو سليمة و تم التشخيص إكلينيكيا وبإستخدام تفاعلالبلمرةالمتسلسل للنسخ العكسي الكمى-(real time RT-PCR) وتضمنت الأعراض الإكلينيكية الإسهال الأبيض، والخمول، والريش غير منتظم، والصفة التشريحية التى أظهرت تغيرات باثولوجية فى الكيس الفبراشى وإلتهاب الكليتين، والنزيف في العضلات، والنزيف النقطي عند التقاطع بين المعدةالغدية والقانصة. وكانت معدلات االنفوق تتراوح من 0.31 إلى 25٪. وبإستخدام البلمرة المتسلسل للنسخ العكسي الكمى تم التعرف على فيروس IBDV في 47 من أصل 62 (75.8٪) من القطعان المتوقع اصابتها ولم يتم اكتشاف أي فيروس IBDV في القطعان السليمة ظاهريا. كماتم تسجيل معدلات إنتشار عالية في الدجاج بعمر 18-20 يومًا ومن سلالات ساسو وإنديان ريفر وهوبارد. أظهرت قطعان الدجاج المحصنة باللقاح المتوسط الحى(Nobilis Gumboro D78) فقط، واللقاح المتوسط + المتوسط بلس، وVaxxitek-ND-IBD، وInnovax-ND-IBD معدل اكتشاف بنسبة 100%. ومن اللافت للنظر أن برنامج اللقاح الأكثر فعالية كان في القطعان التي استخدمت اللقاح الوسيط +Vaxxitek-ND -IBD(33.3%). يمكن أن نستنتج أن لقاحات IBDV الحالية المطبقة بجرعة واحدة توفر حماية غير كافية ضد سلالات فيروس مرض التهاب جراب فابريشيا المعديIBDV خاصة في السلالات الحية بسبب تداخل الأجسام المضادة الأمومية. وفي الوقت نفسه، فإن التطعيم باللقاح المؤتلف متبوعًا بجرعة أو جرعات معززة من اللقاحات الحية يوفر حماية جيدة ويمنع عدوى IBDV. ولذلك، فمن الضروري الاستفادة من نتائج أفضل تجارب التطبيق الحقلي لتحديث وتحسين برامج التحصين ضد فيروس مرض التهاب جراب فابريشيا المعديIBDV مع الأخذ في الاعتبار ممارسات الأمن الحيوي في مزارع الدجاج.مع دارسة متعمة للتعرف على التتابع الجينى الكامل للفيروس لتحديد العترات المتحوة والجديدة.