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RESEARCH ARTICLE

Role of Emergency Vaccination as a Trial to Control DEV Infection in Muscovy Ducklings

Tamer M. Abdullatif[1], Reham M. ElBakrey[1*], Mervat A. Ayoub[2] and Ibrahim A. Ghanem[1]

[1] Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia, 44511, Egypt.

[2.] Department of Pathology, Animal Health Research Institute, Dokki, Zagazig Branch, Egypt.

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Abstract

Duck viral enteritis (DVE) is an important viral disease affects ducks all over the world. Vaccination is an effective way to control DVE, and an attenuated vaccine is used extensively worldwide. In this study, we attempted to control the disease by emergency vaccination. Three out of four groups (group 1, 2 and 3) of 21 days old Muscovy ducklings were infected with 0.5 ml of $10^{5.5}$ duck embryo lethal dose 50 ($dELD_{50}$/ml) of identified field duck enteritis virus (DEV) isolate. After the onset of clinical signs (6 days post infection), two out of three infected groups (group 2 and 3) were vaccinated with live attenuated vaccine using oral and S/C routes, respectively. Birds of infected- non vaccinated group (group1) showed the characteristic clinical and pathological features of the disease at the fifth day post infection that progressed throughout 21 days of the experiment, with 33.3% mortality rate. The severity and frequency of clinical and pathological findings in infected-vaccinated groups (group 2 and 3) were relatively lower than those of group1. Only 13.3% mortality rate was recorded in group 2; moreover no death was detected in group 3. The presence of DEV in the tissues collected from dead and euthanized live ducklings at the end of experiment was confirmed by PCR. Our results showed that the use of commercial live attenuated vaccine via S\C route as an emergency vaccine could ameliorate the clinical and pathological findings of the disease and considered as an effective tool to control DEV infection.

Keywords: Duck Viral Enteritis; Vaccine; Ducklings; Route.

Introduction

Duck viral enteritis (DVE) is one of the most duck disease threatened duck industry [1]. It is an acute, sometimes chronic, contagious, lethal and systemic infectious disease of waterfowl caused by duck enteritis virus (DEV) [2, 3]. It causes high losses in commercial duck industry [4-7]. DEV was classified as a member of family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Mardivirus* as *Anatid Herpesvirus 1* denoted after the host family *Anatidae* [8].

The disease is distinguished by damage of vascular systems, hemorrhages on tissues and eruptions on digestive mucosa, besides lesions in lymphoid organs and degenerative changes in parenchymatous organs [9, 10]. It is difficult to control duck enteritis virus because it can establish an asymptomatic carrier state in waterfowl (latent infection) and it is detected only during periods of intermittent virus shedding [11]. It is thought that reactivation of latent virus is responsible for the transmission of the virus in between waterfowl population and occurrence further outbreaks [12].

Immunization of ducks is an efficient tool to prevent DEV infection [13, 14]. Live attenuated and/or an inactivated vaccines induced effective immune response in birds [12]. DEV attenuated live vaccine has been used extensively worldwide and provides a good protection [14]. However, Dardiri [15] found that the high level of serum antibody

***Corresponding author e-mail: (rehamemara3@gmail.com),Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia 44511, Egypt.**

does not prevent virus shedding from the cloaca or esophagus of infected waterfowl. Bordolai *et al.* [16] mentioned that sometimes this vaccine fails to protect the ducks despite regular vaccination; this might be due to low titer and poor immunogenicity. Among all control efforts, Wang *et al.,* [17] explained that emergency vaccinations of the threatened duck flocks are considered as one of tools to control the DVE outbreak.

Thus, the present study was planned as an attempt to control DEV infection in experimentally infected Muscovy ducklings using commercial live attenuated DEV vaccine as an emergency vaccine through different routes.

Materials and Methods

Ethics Declaration

The experiment was approved by Institutional Animal Care and Use Committee of Zagazig University with approval number ZU-IACUC/ 2/F/114/2018. Experiment was carried out in agreement with the approved guidelines.

Birds

A total of sixty, 21 days old Muscovy ducklings were obtained from private farm in Sharkia Governorate with history of no vaccination against DEV. All ducklings were reared under strict hygienic conditions with suitable temperature and relative humidity. The birds were divided into 4 equal groups (1, 2, 3 and 4), 15 birds/ group and reared in experimental units, Faculty of Veterinary Medicine, Zagazig University.

Virus

Duck enteritis virus field strain was isolated from backyard duck flock in Sharkia, Egypt.

It was identified by PCR using DNA polymerase gene. The virus was propagated in the CAM of 10-13 days of embryonated Muscovy eggs. The duck embryo lethal dose ($dELD_{50}$) was determined by the method of Reed and Muench, ($10^{5.5}$ $dELD_{50}$/ml) [18].

This virus strain was used as infected virus with dose of 0.5ml via intramuscular (I/M) route to investigate its pathogenicity in Muscovy ducklings and evaluate the efficacy of commercial live attenuated DEV vaccine as emergency vaccine.

Vaccine

Commercial live attenuated DEV vaccine was used as emergency vaccine and is commonly used in Egyptian field. This vaccine was obtained from Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt. It was prepared from Jansen strain with a titer of $10^{7.6}$ EID_{50}/ml, Batch no: 10, and is given with dose of 0.5ml S/C or I/M. It was proved to be valid and passed all the evaluation tests.

Experimental design

Ducklings of the first three groups (1, 2 and 3) were infected with 0.5 ml of $10^{5.5}$ $dELD_{50}$/ ml of DEV field strain via I/M route. The emergency vaccination was carried out in groups 2 and 3 with dose 0.5ml of commercial vaccine using (oral and S\C) route respectively after the onset of clinical signs; at 6 days post infection (27 days old). Ducklings in group 1 and 4 were kept as positive control infected-non vaccinated group and negative control non-infected non vaccinated group, respectively (Table 1).

Table (1): Experimental Design of emergency vaccination of duck enteritis virus (DEV) in Muscovy ducks

Duck groups	Infection	Emergency vaccination
	21 days old	**27 days old**
1		-
2	$10^{5.5}$ $dELD_{50}$ of field viral strain with dose of 0.5ml I/M	Commercial live attenuated vaccine via oral route with dose of 0.5 ml
3		Commercial live attenuated vaccine via S/C route with dose of 0.5 ml
4	-	-

Ducklings were observed twice daily throughout the period of experiment (21 days) for clinical signs and/or deaths. Necropsy was performed on all dead and sacrificed live ducklings at the end of experiment to examine gross lesions. Different organs were collected separately and prepared for virus detection using PCR and histopathological examination.

Histopathological examination

Selected organs; liver, intestine, caecum, spleen, thymus, bursa, and brain were fixed in 10% neutral buffered formalin solution, routinely processed and embedded in paraffin. Paraffin sections were prepared and stained with hematoxyline and eosin then examined microscopically for histopathological finding [19].

Polymerase chain reaction

Viral DNA was extracted from collected organs (intestine, liver and brain) using DNA extraction kit (viral Gene-Spin™, Cat#17151, iNtRON Biotechnology, Inc), following the instructions of the kit manufacturer. The primers used for DNA polymerase gene amplification are Forward 5´-GAAGGCGGGTATGTAATGTA-3´ and Reverse 5´-CAAGGCTCTATTCGGTAATG-3´ as described by Wu et al. [20]. A 25µL reaction mixture was carried out using 2x PCR master mixture solution (*i*-Taq™) (Cat#25027, iNtRON Biotechnology, Inc). Thermal condition used for the amplification of DNA polymerase gene was: initial denaturation at 94°C for 2 min; followed by 35 cycles of reaction comprising with 94°C for 1 min, 56°C for 1min, 72°C for 2 min, with a final extension at 72°C for 7 min. Then, the products of PCR were loaded to the appropriate well of the 1% agar gel. After electrophoresis, the DNA was visualized using UV trans-illuminator.

Results

Clinical signs and gross findings in ducklings group infected with DEV field strain

Typical clinical signs and post-mortem lesions of DEV were observed in ducklings of group 1 that intra-muscularly infected with field DEV strain. Five days post infection typical clinical and post mortem lesions were observed on up to 70% of infected ducklings as anorexia, depression, nasal and ocular discharge, dehydration, increased thirst, watery and sometimes greenish diarrhea. At around 6 days p.i, respiratory signs with nasal and ocular discharge and dark brownish mucoid diarrhea were observed. The first mortality case with nervous signs; ataxia and tremors of head and neck before death was recorded at 9 days p.i. Bloody diarrhea was noticed in 66.7% of infected ducklings at 13 days p.i. (Figure 1). The recorded mortality rate in the infected group was 33.3%.

Necropsy of dead and euthanized survived ducklings revealed typical lesions of DEV, including inflamed and enlarged intestinal annular bands, severe hemorrhagic enteritis especially in rectum with sloughing of the entire mucosa. Caecum was filled with bloody and then cheesy cecal core. Liver was enlarged with petechial hemorrhages and/or white necrotic foci on the surface. Moreover, the enlarged and mottled spleen and congested kidneys were detected. Petechial haemorrhags were showed on epicardium and coronary fat of the heart. The primary lymphoid organs especially bursa and thymus were inflamed, hemorrhagic with presence of whitish yellow exudate in the lumen of bursa. Also, congestion and edema with petechial haemorrhage on the cerebral hemisphere was seen in brain of infected birds (Figure 1).

The incidence of clinical signs and post mortem lesions recorded in the infected ducklings group during the period of experiment were recorded (Figure 2).

Clinical signs and gross findings in infected-vaccinated duckling groups

After emergency vaccination within 3-4 days, ducklings of groups 2 and 3 showed normal activity and viability especially in group 3. Mild clinical signs were showed in birds as; respiratory signs and watery diarrhea. Additionally bloody diarrhea was observed in 13.3% of group 2 only at 7 days post vaccination (Figure 2A). The mortality rate

reached 13.3% in group 2 while no deaths were recorded in group 3.

Necropsy of ducklings in groups 2 and 3 was performed and detected that no specific gross lesions were observed in brain, bursa, thymus or kidneys of ducklings in group3 but a mild lesions were seen in intestine as; hemorrhagic enteritis and inflamed and hemorrhagic rectum with percentage of 13.3% and 6.7%, respectively. Also, lesions were showed in liver (6.7%), spleen (13.3%) and heart (6.7%) of ducklings. While in group 2 some characteristic DEV lesions were noticed (Figure 2B). The severity and frequency of these lesions in birds of group 2 and 3 were relatively lower than in infected- non vaccinated group. No specific clinical signs or gross findings were observed in non-infected-non vaccinated control ducklings (group 4)

Histopathological analysis

Pathological changes and its severity were reported in various tissues in groups 1, 2 and 3 (Figure 3) and (Table 2).

Digestive tract: the intestine showed necrotic enteritis with present of large number of desquamated cells and leucocytes in the lumen. Massive infiltration of leucocytes was seen in mucosa and submucosa. The mucosal layer of the intestine was replaced with lymphocytic aggregation and syncytial cell formation in epithelial lining villi. While, in group3, metaplastic and hyperplastic of intestinal epithelial lining was observed. In caecum; all groups showed necrosis of villus epithelium, necrotic epithelium adhered with inflammatory cells in lumen, in addition to congestion of cecal blood vessels in lamina propria and submucosa.

Brain: neuronophagia, edema, degenerative changes were detected which were severe in group1 and mild in group 3. Besides, cerebral blood vessels in birds of groups 1 and 2 were dilated and engorged with blood and surrounded by perivascular edema.

Liver: nearly in all groups; focal area of coagulative, necrosis degenerative changes mainly cloudy swelling, vacuolar degeneration and fatty changes were showed. As well as eosinophilic intra- nuclear inclusion bodies (IN\IB), congestion of hepatic blood vessels and infiltration of portal area with inflammatory cells were seen.

Spleen: congestion of splenic blood vessels and multifocal necrosis of the lymphoid elements of the white pulp were detected mainly in group 1. Additionally, perivascular edema, vaculation of tunica media of splenic blood vessels were observed in group2. Depletion of lymphocytes with congestion of splenic blood vessels was seen in group 3.

Bursa: group 3 not showed any changes. While, groups 1 and 2 showed depletion of lymphocytes, in addition to hyperplasia of epithelial lining, and eosinophilic IN\IB in a variable number of bursal cells in group 1.

Thymus: group 3 not demonstrated any changes. Although, in groups1 and 2 showed depletion of lymphocytes and congestion of thymic blood vessels.

Table (2): Microscopic lesions in the visceral organs of Muscovy ducklings in different experimental groups exposed to emergency vaccination of duck enteritis virus (DEV)

Groups	Severity of lesions in the visceral organs							
	intestine	Caecum	Liver	Spleen	Bursa	Thymus	Brain	Heart
(1) Infected group	+++	++	+++	+	+++	+++	+++	+++
(2) Infected-emergency vaccinated (oral route)	++	+	++	+	+	+	+++	++
(3) Infected-emergency vaccinated (S\c route)	+	+	+	+	N	N	+	+

Severity of lesion in different organ: +++= severe; ++=moderate; +=mild; N =normal

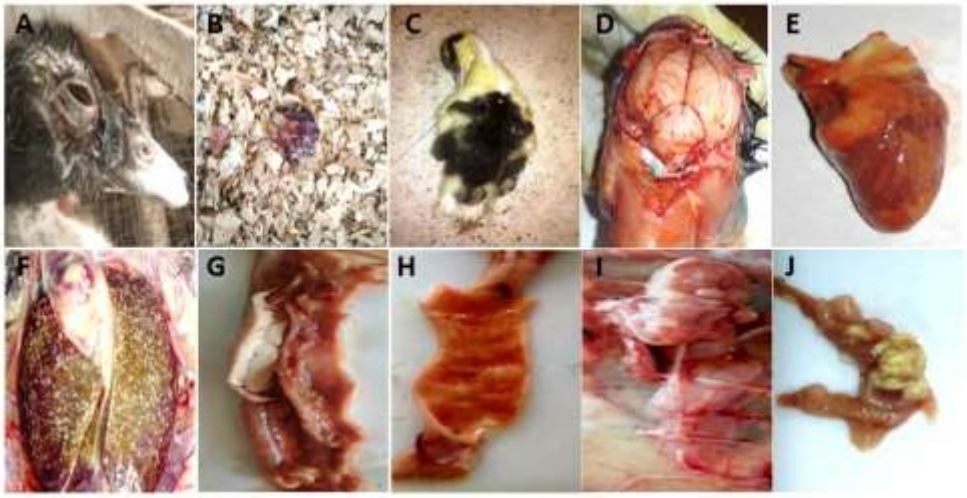

Figure 1: Clinical signs and gross lesions of the duck enteritis virus (DEV)-infected ducklings. (A) Excessive ocular discharge with pasted eye, the feathers around the eyes are damp. (B) Bloody diarrhea. (C) Nervous signs in form of tremors in head and neck. (D) Congestion and punctate hemorrhages on brain. (E) Hemorrhages on heart. (F) Enlarged liver with blood spots and necrotic foci. (G) Enteritis with necrotizing pseudo membranous mucosal lesions in duodenum. (H) Haemorrhages on mucosa of rectum. (I) Slight congestion in thymus. (J) Inflamed bursa with presence of whitish yellow diphtheritic exudate in the lumen.

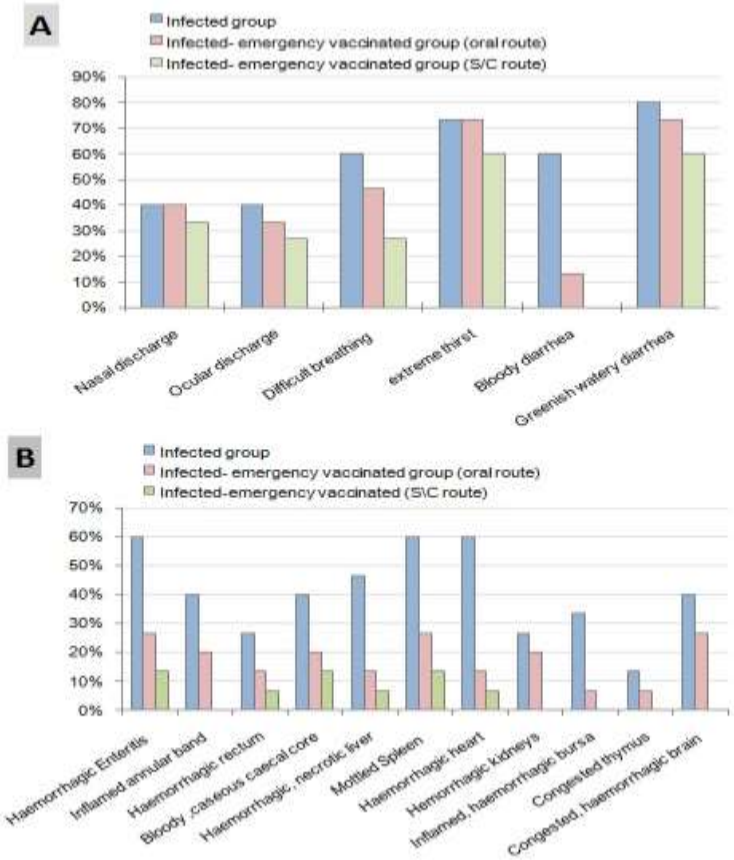

Figure 2: Incidence of clinico-pathological findings among infected and infected-vaccinated duckling groups with duck enteritis virus (DEV) throughout the experimental period. (A) Incidence of clinical signs; (B) Incidence of post-mortem lesions.

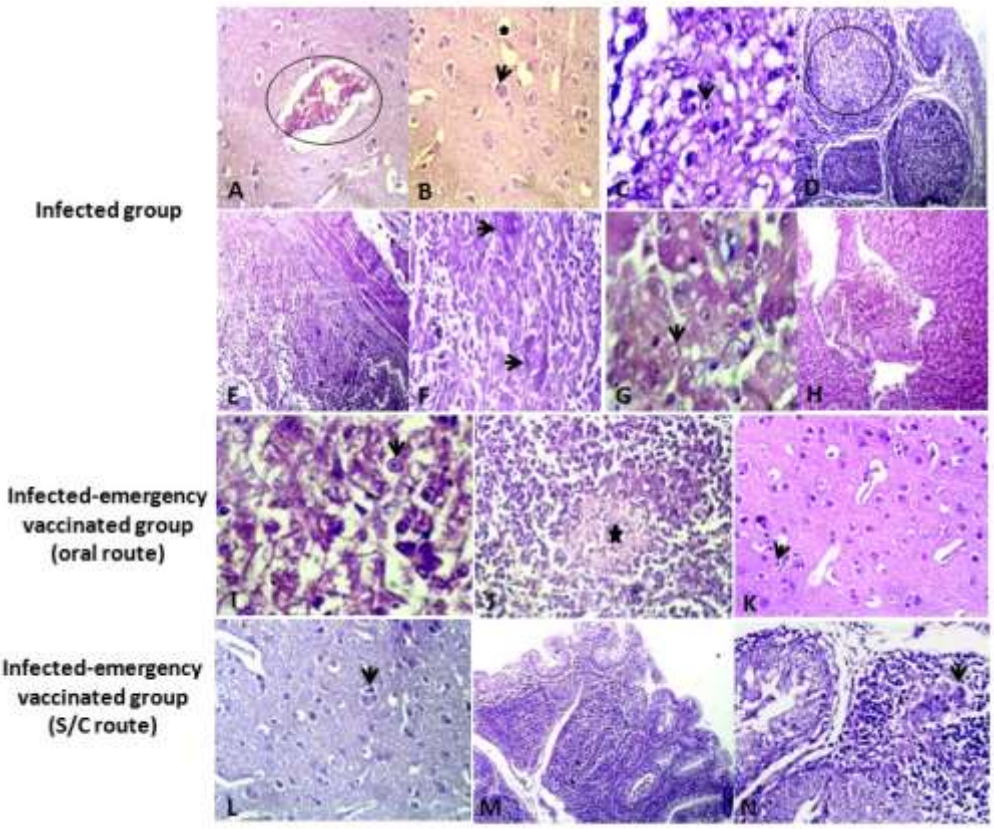

Figure 3: Histopathological changes in different tissues of experimentally infected duckling groups with duck enteritis virus (DEV). (A) Brain showing congested cerebral blood vessels and perivascular edema (circle) (H&E X400). (B) Brain showing neuronophagia (arrow), edema (star) (H&E X400). (C) Bursa showing eosinophilic Intra- nuclear inclusion bodies (IN\IB) in some degenerated columnar epithelium lining (arrow) (H&E X1000). (D) Bursa showing coagulative necrosis (depletion) at center of lymphoid follicles (Small circle) (H&E X400). (E) Intestine showing necrotic enteritis represented by necrosis of the villus epithelium, presence of large number of desquamated cells and leucocytes in the intestinal lumen (H&E X100). (F) Intestine showing syncytial cell formation in epithelial lining villi (arrow) (H&E X400). (G) Liver showing degenerative changes in some hepatocytes mainly cloudy swelling, vacuolar degeneration and fatty changes with eosinophilic intra-nuclear inclusion bodies (IN\IB) (arrow) (H&E X1000). (H) Liver showing leukocytic infiltration of hepatic sinusoids, congestion and edema in portal area with extravasation of erythrocytes (H&E X100). (I) liver showing eosinophilic IN\IB within nucleus of some degenerated hepatocytes (arrows) (H&E X1000). (J) Spleen showing coagulative necrosis (depletion) of the lymphoid elements of the white pulp (star-circle) (H&E X1000). (K) Brain showing degenerated neurons, satellitosis and neuronophagia (arrow) beside area of edema around congested cerebral blood vessel (H&E X400). (L) Brain showing degenerated neurons, satellitosis and neuronophagia (arrow) (H&E X400). (M) Intestine showing hyperplastic and metaplastic changes of epithelial lining mucosa, infiltration of lamina propria and submucosa by inflammatory cells (H&E X100). (N) Intestine showing leucocytic infiltration with syncytial cell formation in epithelial lining villi (arrow) (H&E X400).

Molecular re-confirmation of DEV in infected ducklings

DNA polymerase gene of DEV was amplified from the tissues (intestine, liver and brain) that collected from freshly dead (5 birds in group 1 and 2 birds in group 2) and from sacrificed live ducklings of 4 groups (2 birds/ group) at end of the experiment. The PCR products were showed as a band at 446-bp in tissue samples collected from groups 1, 2, and 3. While the tissue samples of non-infected-non vaccinated group (group 4), the results of PCR were negative (Data not showed).

Discussion

Duck viral enteritis is an acute highly contagious disease, distributed all over the world. The disease caused by herpes virus infection of Anseriformes and has caused

substantial economic losses to duck productions [21]. This study was designed to investigate the efficacy of commercial live attenuated DEV vaccine that used as emergency vaccine to control DEV infection in 21 days old Muscovy ducklings.

The clinical pictures of the disease were noticed at the 5^{th} day post infection and characterized with symptoms of ocular nasal discharges and mucoid bloody diarrhea with nervous signs. These signs were extended and progressed in the group1 (infected-non vaccinated group). The typical clinical symptoms were previously described by many authors [22- 26]. While the signs were relatively less in groups 2 and 3, the bloody diarrhea or nervous signs were not noticed in group 3. Additionally, activity and viability in group 3 were better than group1 and 2. Moreover, the recorded mortality rates were 33.3% in group 1, 13.3% in group 2 and 0% in group 3. This could be attributed to the vaccine ameliorate the clinical signs and mortality rates in infected-vaccinated groups especially in group that vaccinated via S/C route. The ducklings in non-infected- non vaccinated group (group 4; control negative group) were healthy and no clinical signs or mortalities were seen throughout 21 days of the experiment.

Regarding to the pathological lesions, the virus produced consistent gross and histological lesions as supported by [11, 23, 24, 26-28]. Among these post mortem and microscopical lesions; congestion, hemorrhages, necrosis and degenerative changes that commonly observed in wide range of organs and tissues especially in digestive tract, heart and liver. These indicated that DEV is a pantropic virus and has broad tissue tropism which replicates rapidly in a variety of tissues and causes severe pathological lesions as described by Li *et al.* [26]. Also, DEV replicates in the vascular endothelial cells and causes severe hemorrhages and degenerative changes in different organs [29]. The frequency and severity of these lesions in infected-vaccinated groups (groups 2 and 3) were relatively lower than that in infected non-vaccinated group.

Eosinophilic intra-nuclear inclusions have been seen in a variable number of cells as bursal cells and hepatocytes. The detection of inclusion bodies had diagnostic significance for DEV infections. Salguero *et al.* [30] pointed to histopathological findings as enteritis, multifocal necrotic with intra-nuclear inclusion bodies are characteristic of herpes virus infection and are useful in confirming the infection with DEV.

Furthermore, lesions of the lymphoid tissues including bursa, thymus and spleen that detected in group 1 and 2 were congruous with other previous studies where DEV has been caused lymphoid organ lesions and resulted in severe immunosuppression in infected birds, which indicated that lymphocytes of the lymphoid and intestinal tissues are the main targets for DEV infection [30-34]. These lymphoid lesions were not detected in group 3 and this could be referred to the role of vaccine that could counteract the broad tissue tropism nature of the virus and improve the immune responses against infection with DEV.

Through clinical and pathological pictures that were examined under the experimental infection, infected-emergency vaccinated groups had clinico-pathological pictures lower than in infected- non-vaccinated group especially that vaccinated via subcutaneous route. This explained by Huang *et al.* [14] who demonstrated that subcutaneous administration of the attenuated DEV vaccine could produce efficient humoral, cellular, and mucosal immune responses against DEV challenge. Also, Xiaoyan *et al.* [35] proved that oral immunization with live attenuated DEV vaccine is effective in inducing mucosal immunity that is important in protecting animals from intestinal infections.

Cell mediated immunity play an important role in recovery from the infection due to the intracellular nature of herpes virus infections as previously reported [36]. Moreover, Huang *et al.* [14] found that cell-mediated and mucosal immunity are important in protection

against Duck Enteritis virus and high level of humoral immunity doesn't prevent virus shedding.

Conclusion

From the above results it can be concluded that under experimental condition, using of commercial live attenuated vaccine as an emergency vaccination succeed in reducing the severity and frequency of clinico-pathological lesions, in addition to decrease mortality rate which reached to zero in group that vaccinated via subcutaneous route. Consequently, emergency vaccination could be used as an effective tool in controlling DEV infection. We still in need further studies to understand the type of immune responses to the live attenuated DEV vaccine through subcutaneous and oral immunization in ducks.

Conflict of interest

The authors declared that they have no conflict of interest.

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الملخص العربي

دور التحصين الاضطراري كمحاولة للسيطرة على العدوى بالتهاب الأمعاء الفيروسي في البط المسكوفي

تامر محمود عبداللطيف[١], رهام محمد البكرى[١]*, ميرفت عبدالبديع أيوب[٢]، إبراهيم عبدالرحمن غاتم[١]

[١] قسم طب الطيور والأرانب- كلية الطب البيطرى- جامعة الزقازيق- مصر

[٢] قسم الباثولوجيا- معهد بحوث صحة الحيوان- الدقى- فرع الزقازيق- مصر

يعد إلتهاب الأمعاء الفيروسي للبط (DVE) مرضًا فيروسيًا مهمًا يصيب البط في جميع أنحاء العالم. فالتحصين يعتبر أداة فعالة للسيطرة على العدوى، واللقاح الحي هو اللقاح الروتيني الرئيسي المستخدم. في هذه الدراسة، تمت محاولة للسيطرة على المرض عن طريق استخدام التحصين الاضطراري. في ثلاثة مجموعات من أصل أربعة من البط المسكوفى البالغ من العمر ٢١ يوم تمت إصابتهم بجرعة $10^{5.5}$ $dELD_{50}/ml$ بالعترة المعزولة من الحقل. وبعد يوم واحد من بداية ظهور العلامات السريرية (بعد ستة أيام من العدوى)، تم تحصين مجموعتين من بين الثلاث مجموعات المصابة (امجموعتى ٢ و٣) باللقاح الحي باستخدام طريقة الحقن في الفم وتحت الجلد على التوالي. الطيور بالمجموعة المصابة الغير محصنة (المجموعة الاولى) قد أظهرت خصائص سريرية ومرضية مميزة للمرض في اليوم الخامس بعد الإصابة والتي تطورت خلال فترة التجربة، وكانت معدلات النافق ٣, ٣٣ ٪. بينما كانت وتيرة وشدة الاعراض المرضية في المجموعات التي تم تحصينها (مجموعتى ٢ و٣) أقل نسبيا من تلك الموجودة بالمجموعة الاولى. تم تسجيل معدل نافق ٣، ١٣ ٪ فقط في المجموعة الثانية؛ علاوة على ذلك، لم يتم تسجيل أي نافق بالمجموعة الثالثة. وقد تم التأكد من وجود الفيروس في الأنسجة المجمعة من البط النافق والمذبوح في نهاية التجربة بواسطه اختبار البلمرة المتسلسل. هذه النتائج قد أظهرت أن استخدام اللقاح الحي التجاري عن طريق الحقن تحت الجلد كلقاح اضطراري يمكن أن يخفف الأعراض السريرية والمرضية للمرض ويعتبر كأداة فعالة للسيطرة على عدوى التهاب الأمعاء الفيروسي بالبط.