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COMPARATIVE PATHOLOGICAL AND IMMUNOLOGICAL STUDIES ON DIFFERENT OIL ADJUVANATED INACTIVATED NEWCASTLE DISEASE VIRUS VACCINES (NDVV) PRIVATE

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SUMMARY

The present study was applied to evaluate the physical properties of ISA-70 Marcol-52 and paraffin oil adjuvant used in commercial vaccines and describes the pathological changes and serological response of chickens after intramuscular injection.

A total of 140 three - week old white Leghorn chickens, specific pathogen free were obtained from Koum Osheim, Fayoum Governorate. Chickens were divided into four equal groups one of them is control, the other three groups were injected intramuscularly with the following oil adjuvant emulsions containing inactivated Newcastle disease virus (NDV) antigen: ISA-70, Marcol-52 and paraffin. Five chickens of each group were sacrificed at 72 hours and at 1, 2, 4, 8, 12 and 16 weeks post-vaccination.

The pathological reaction induced by ISA-70 emulsion was characterized by focal aggregation of macrophages, lymphocytes, epithelioid cells and fibroblasts around the small cysts in muscle. These lesions peaked at 2 weeks post-injection and considered as mild pathological changes if compared with Marcol-52 and paraffin oil adjuvant.

The serological findings revealed that the enhancing effect of ISA-70 on NDV antigen was more than that of Marcol-52 and paraffin oil adjuvant.

INTRODUCTION

Newcastle disease (ND) is highly contagious disease and attempts to control it by slaughter; sanitary measures and quarantine are often unsuccessful. When it becomes endemic, vaccination of flock at risk is highly effective method of control (Cross, 1988).

Oil adjuvants have been applied to inactivated vaccines against several avian diseases such as Newcastle disease (ND) (Aitken and Survashe, 1974 and Bennejean et al., 1978). The value of inactivated oil emulsion vaccines for avian viral diseases has been demonstrated by production of a high level of circulating antibodies and long - term protection against challenge with virulent strain (Box and Furminger, 1975).

The major advantages of inactivated vaccines are the very low level of adverse reactions in vaccinated birds. The ability to use them in situations unsuited for live vaccines, especially, if complicating pathogens are present and extremely high level of protective antibodies of long duration is desired (Mohammadi et al., 1996).

The local tissue reaction induced by these oil adjuvant vaccines has not been described in chickens in detail. In a previous paper, pathological changes at intramuscularly injection site were recorded in guinea pigs and rats induced by the new oil adjuvant ISA-70 was characterized by granulomatous lesions consisting of cysts, macrophages, small round cells and fibrous connective tissue proliferation (Yamanaka et al., 1992).

The present study compared the physical properties of ISA-70 emulsion with those of Marcol-52 and paraffin oil adjuvant. Serological and pathological examination of chickens intramuscularly injected with different oil adjuvant emulsions containing inactivated NDV antigen were elucidated.

MATERIALS AND METHODS

Chickens:

Three-week-old white Leghorn chickens, specific pathogen free (SPF) obtained from Koum Osheim, Fayoum Governorate were used. They were housed in batteries and fed on a balanced basal diet.

Antigen preparation:

The F strain of NDV antigen was propagated in chicken embryos and the allantoic fluid. The infectivity titer of the virus before inactivation was $10^{8.5}$ EID_{50}(egg infective dose) per 0.1 ml. The inactivation of virus was carried out using 0.2% formalin.

Vaccine Preparation:

The whole procedure of vaccine production was carried out under a Lamin Air flow unit. The vaccine was stored at 4°C.

1- Montanide ISA-70:

A water-oil emulsion adjuvant vaccine was produced mechanically by using a homogeniser, according to the protocol of Seppic Pharmacy Division, France.

2- Marcol-52:

The preparation of water-oil emulsion adjuvant of Marcol-52 was done according to Thayer et al. (1983).

3- Paraffin:

Paraffin oil adjuvant vaccine was prepared, according to Brugh et al. (1983) .

Evaluation of the physical properties of oil emulsions:

The physical properties of the prepared oil emulsion vaccines were investigated as following:

1- Emulsion type:

The type of emulsion was determined by the drop test according to (Stone et al., 1978).

2- Relative viscosity:

It was determined according to Cessi and Nardelli (1973).

3- Emulsion stability:

It was experessed as weeks of storage time during which the oil and aqueous phases did not separate.

Toxicity test:

It was done on the new oil adjuvant ISA-70 according to Berlin (1962) as following:

Intraperitoneal injection of 0.25 ml of Montanide ISA-70 into 10 Swiss male mice proved to be free from any specific pathogenic organism. The animals were weighed regularly during the experimental period (8 days) and then dissected for examination of the main organs. The test is satisfactory if there were absence of peritonitis and difference in weight gain with control animals less than 10.5%.

Experimental Design:

A total of 140, twenty-one days old chickens were divided into four groups of 35 birds each, one of them is control and the other three groups were injected intramuscular with 0.5ml of the prepared vaccines with different oil emulsions separately. Five chickens of each group were sacrificed at 72 hours and at 1, 2, 4, 8, 12 and 16 weeks post-injection (PI). Serological and pathological examinations were done to evaluate the potency of Newcastle disease (ND) vaccine prepared in three types of oil adjuvant (ISA-70, Marcol-52 and paraffin).

Quality control:

The control tests included sterility, safety and potency evolution.

1- Sterility test:

Antigen was tested for bacterial and fungal contaminants. The final product underwent the same chicks.

2- Safety test:

A group of 25 SPF chickens were inoculated

with the prepared vaccine and were observed for a period of 15 days for any possible untoward manifestations.

3- Potency test:

A group of 25 SPF chickens were vaccinated, one dose/bird and were bled regularly for detection of changes in the serum antibodies, measured by haemagglutination inhibition test for twenty one day from the vaccination then challenged by a highly virulent strain of NDV.

Challenge Test:

Vaccinated birds separately with unvaccinated (control) chickens were challenged by intramuscularly injection with strain of (NDV)$10^{5.5}EID_{50}$.

Histopathological examination:

Samples were taken from the injection site (muscle) of tested chicken after 72 h, 2, 4, 8, 12&16 weeks PI. They were fixed in 10% neutral buffered formalin and embedded in paraffin by standard methods. Sections were stained with haematoxylin and eosin H & E according to Bancroft (1996).

Serology:

Serum samples were obtained at 72 hours and 2, 4, 8, 12 and 16 weeks PI for haemagglutination and haemagglutination inhibition (HI) tests.

1-Haemagglutination test (HA):

This was carried out according to the method described by Allan and Gough (1976) using microtitre plates. The dilution in the well-showed 100% agglutination was taken as the titer.

2-Haemagglutination Inhibition (HI) Test:

Standard method was employed, using 4 HA units and 1% v/v chicken red blood cells. The HI titers were seen in the highest dilutions of sera causing complete inhibition of 4 HA units.

RESULTS

Physical properties of oil emulsion:

Oil emulsions prepared with ISA-70 were miscible in mineral oil but not in water. The viscosity of ISA-70 emulsions at 24°C was lower than that of Marcol and paraffin adjuvant

Toxicity test:

It was done on the new oil adjuvant by intraperitoneal injection of ten Swiss male mice with 0.25 ml of ISA-70. The animals were weighed regularly during 8 days post-injection and the difference in weight gain with control mice less than 10.5%. All animals were scarified for post mortem examination, which revealed absence of peritonitis.

Quality control:

All vaccines prepared with the different oil adjuvant were free from any contaminant and potent but ISA-70 more potent as measured by HI test.

hological changes at the injection site:

le (1) compared the histopathological changes he injection site induced by the different adju- it.

Adjuvant ISA-70:

The subcutaneous tissue and the superficial thigh muscles of chickens injected with ISA-70 emulsion containing NDV antigen showed remnants of the inoculum with slight oedema at 72 hours PI and first week PI.

Microscopically, mild acute focal exudative inflammatory changes characterized by leukocytic infiltration of macrophages, lymphocytes and small numbers of heterophils were observed in the thigh muscle and subcutaneous tissues (fig. 1A).

Numerous oil droplets that in some areas form cyst like spaces of varying size were evident in the muscles at 1 to 2 weeks post injection (fig. 1B). Granulomatous lesions consisted of epithelioid cells; macrophages and fibroblasts were seen around the cysts (fig. 1C). Focal aggregation of plasma cells and lymphoid cells between the muscle fibers at the site of injection were observed. These cell reactions were regressing by week 4 PI and the cysts tended to decrease in number and appeared to be reduced in size (fig. 1D).

At 12 to 16 weeks PI, only minute cysts with small number of epithelioid and plasma cells were observed between the muscle fibers.

2. Marcol-52:

In the chickens injected with Marcol oil emulsion containing NDV antigen, yellowish caseous substances were localized between the muscles of the thigh at 72 hours and persisted up to 4 weeks PI.

Microscopically, at 72 hours, moderate inflammatory reaction at the site of injection, which was infiltrated mainly by heterophils and macrophages with intermuscular oedema (fig. 2A).

At 1 to 2 weeks PI, granulomatous reaction was characterized by proliferation and accumulation of macrophages, lymphocytes, epithelioid cells,and fibroblast with caseous area. Multiple cysts surrounded by small round cells and formation of lymphoid follicles also were recognized.

These reactions regressed during weeks 4 to 8 PI. At week 16 PI, there were no lesions at the injection sites.

3. Paraffin:

Chickens injected with paraffin oil emulsion containing NDV antigen showed remnants of the inoculum between the superficial deep thigh muscles at 72 hours PI.

At 1 to 4 weeks PI, yellowish white cheesy substances were seen between muscles of the thigh. Yellowish discoloration of the muscle at the injection site was also observed at 1 week PI and persisted for at least 12 weeks PI.

Table (1): Main histopathological changes at injection sites in chickens injected with different oil adjuvant.

Time PI	Histopathological Changes	ISA-70	Marcol-52	Paraffin
72hours	Acute inflammation	+	+	++
	Abscess	-	-	-
1 week	Acute inflammation	-	±	+
	Cyst formation	++	+	+
	Abscess	-	-	+
	Plasma cells	+	+	+
2 weeks	Cyst formation	++	+	+
	Granulomatous reaction	+	++	+++
	Abscess	-	-	+
	Plasma cells	++	++	+
4 weeks	Cyst formation	+	+	++
	Granulomatous reaction	+	+	++
	Abscess	-	-	+
	Plasma cells	++	+	++
8 weeks	Cyst formation	+	+	++
	Granulomatous reaction	+	+	++
	Abscess	-	-	+
	Plasma cells	+	+	+
12 weeks	Cyst formation	±	±	+
	Granulomatous reaction	±	+	++
	Plasma cells	±	±	-
16 weeks	Cyst formation	±	±	-
	Granulomatous reaction	-	±	+
	Plasma cells	-	-	-

Table (2): Haemagglutination inhibition titres of each group of chickens following a single intramuscular injection of different oil emulsions containing Newcastle disease virus.

Emulsion	HI titres at weeks PI					
	72 hours	2	4	8	12	16
ISA-70	160	686	1280	1040	844	640
Marcol-52	40	343	788	520	394	320
Paraffin	20	320	557	422	343	259

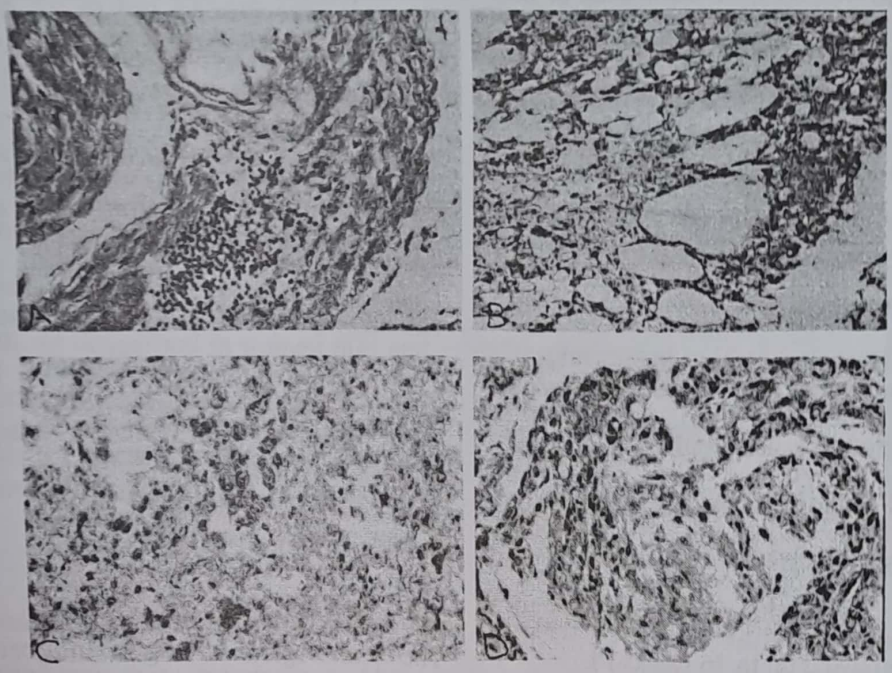

Fig. (1): (A)- Showing mild inflammatory reaction at the site of injection (H&E X200).
(B)-Showing multiple cysts of varied size with lymphoid cells infiltration (H&EX200).
(C)-Showing granulomatous lesion consisted of macrophages, epitheliod cells and fibroblast (H&EX200).
(D)-Showing few number of small size cysts in between the muscle fiber (H&EX200).

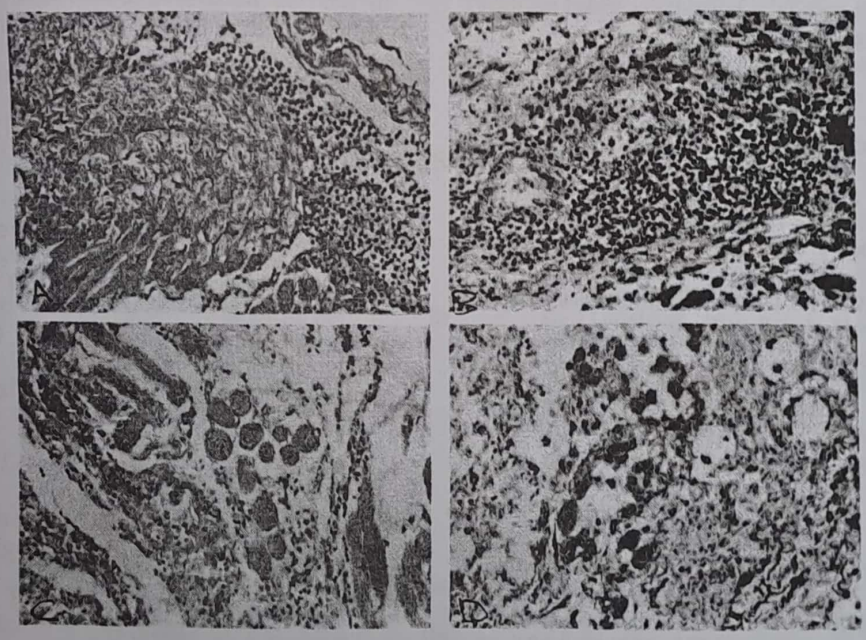

Fig. (2): (A)-Showing moderate inflammatory reaction at the site of injection (H&EX200).
(B)-Showing massive leukocytic infiltration at the site of injection (H&EX200).
(C)-Thigh muscle showing Zenkerís necrosis (H&EX200).
(D)-Showing eiptheliod granulomatous prliferative lesion (H&EX200).

Microscopically, severe exudative inflammatory reaction was observed at 72 hours PI in the muscles characterized by excessive infiltration of heterophils, macrophages and lymphocytes (fig. 2B).

Zenker's necrosis of the thigh muscle and formation of multiple cysts of various sizes were noticed at 1 week PI (fig. 2C).

Epithelioid granulomatous proliferative lesions consisted of necrotic tissues, epithelioid and giant cells with small round cells infiltration, including macrophages, lymphocytes and plasma cells were seen (fig. 2D). These changes peaked at 4 to 8 weeks PI and persisted up to 16 weeks PI.

Serological response in chickens:

Table (2) showed geometric mean NDV-HI antibody titers of each group of chickens following a single intramuscularly injection.

Chickens injected with emulsified ISA-70 containing NDV antigen demonstrated elevation of NDV-HI antibody titer at 2 weeks PI and peaked at (1280) at 4 weeks PI. HI titers persisted at high levels for up to 16 weeks PI.

On the other side chickens injected with emulsified Marcol containing NDV antigen group showed high peak titer (788) and the chickens injected with paraffin oil revealed high peak titer (557) at 4 weeks post injection.

DISCUSSION

The important point for production of oil emulsion adjuvant vaccine is the difficulty of preparing stable water-in- oil emulsions with low viscosity which is essential for assuring injectability and easy handling (Mohammadi et al., 1996).

The oil Adjuvant ISA-70 used in the present study gave a less viscous water -in-oil- type emulsion when compared with Marcol ñ52 and paraffin adjuvant that was prohibited in commercial vaccine products (Stone, 1991).

The pathological changes induced by ISA-70 were characterized by mild to moderate proliferation of epithelioid cells and macrophages around the small cysts scattered between muscle fibers of the thigh and infiltration of small round cells. In addition, there was no evidence of abscess formation. These alterations are in accordance with those given by Yamanaka et al., (1992).

The muscular cysts at site of injection indicated that the antigen enclosed in the water droplets with continuous phase of the oil then it released slowly and provides along stimulation of the immune system (Gupta et al, 1993).

In guinea pigs and rats, pathological changes of ISA-70 emulsion at the site of injection were characterized by granulomatous lesions, small

round cells and fibrous connective tissue proliferation as found by Goto and Akama, (1982).

Chickens injected with ISA-70 emulsion in the present study showed mild acute inflammatory reactions within the first week. The granulomatous reaction was observed in the second week but not so extensive as those described in guinea pigs and rats. These finding indicated that variation in the response to adjuvant among animal species was certain.

Oil-in-water emulsion adjuvant sensitized T cells to release lymphokines that attract macrophages and lymphocytes at the site of injection. Such cellular infiltration was accompanied with an inflammatory reaction that leads to tissue damage. Granulomatous lesions indicated persistent antigen in tissue stimulated the local delayed hypersensitivity, which is characterized by continuous release of lymphokines. The latter leads to accumulation of large numbers of macrophages give rise to epitheloid and giant cells (Stevens and Lowe, 2000). In the present study, ISA-70 induced mild pathological changes if compared with Marcol-52 emulsion. On the other hand, severe tissue reaction of paraffin oil emulsion was characterized by formation of granuloma and abscess.

The serological results obtained in the present study revealed that the enhancing effect of ISA-70 on NDV antigen. Long term persistent of NDV-HI antibody by ISA-70 oil adjuvant was correlated with persistence of histological changes as marked plasma cell and lymphocyte infiltration at the injection site (Yamanaka et al., 1993).

In conclusion, oil adjuvant, ISA-70 gave a very satisfactory result causing desirable immunological responses and mild pathological changes at injection site.

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