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VIRUSES ASSOCIATED WITH CORYZA INFECTION IN CHICKENS

By

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SUMMARY

Isolation of viruses associated with coryza in chicken was carried out. One hundred and thirty eight samples were colleted from 138 flocks representing 851 thousand birds of different ages, breeds and different localities from Sharkia province. The serological examination of sera collected from field cases using HI and AGP tests revealed an incidence of antibodies against ND, IB, ILT Reovirus and Poxvirus in percentage of 7.3, 10.9, 1.5. 4.9 and 0.73 respectively. The viral isolation trials resulted in the isolation of 41 viral agents of which thirteen NDV, sixteen, IBV, three, ILT, two poxvirus and seven reovirus isolates associated with infectious coryza.

INTRODUCTION

Coryza and other respiratory manifestations including swollen head syndrome are common respiratory troubles in poultry production in Egypt. The main possible factors that predispose to more severe and prolonged cause of infectious coryza include intercurrent infection with other pathigens such as the virus of Newcastle, infectious bronchitis, infectious laryngotracheitis, fowl pox and reovirus. bacterial agents like mycoplasma and pasteurella are also factors. In addition, cold, wet conditions could accelerate the disease (Jordan, 1990). To determine the possible incriminated viral agents in the appearane of the clinical signs, mortality and P.M. picture, virus isolation and identification are to be tried to detect the rate of virus infection in this condition. Therefore this investigation was intended to study the possible viral complication for respiratory manifestation in chickens suffering from coryza.

MATERIAL AND METHODS

Examination of birds:

Hundred and thirty eight chikens clinically suffering from coryza were collected from 138 poultry flocks from Sharkia province. Collected birds were subjected to clinical examination.

Experimental birds:

Four hundred and ninety susceptible balady chickens of different ages were used in this study. Age and number of chickens are given in each experiment. The experimental chickens were kept in strict isulation and fed on commercial starter ration. The experimental birds were examined for their fredom before use.

Sera:

One hundred and thirty eight blood samples were collected in sterile tubes from clically affected birds. Sera were separated by centrifugation for 10 minutes at 1500 rpm., and kept at -20°c until used.

Antisera:

Antisera against Newcastle disease virus were kindly supplied by Dr. Sozan Mohammed Health Research Institute, Cairo, Egypt.

Antisera against pox virus , ILT, IB, and reovirus, were obtained from Dr.Amina, A.M. Nawwar, Immunology D epartment, Animal Health Research Institute, Dokki, Cairo ,Egypt

viral antigens:

IB (Beaudetta strain) , ILT (Cover strain), Reovirus (Uconn 1133 strain), and Pox (Connecticut

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strain) as chicken embryo chorioallantoic membrane homogenate were obtained from Dr.Amina A.M. Nawwar, Immunology Department, Animal Health Research Institute, Dokki, Cairo Egypt.

Virus Isolation

Tracheal suspension and nasal swabs diluted (1/10 w/v) was centrifugated for 10 min. at 5000 rpm and the clear suspernatant fluid was treated with antibiotics (10,000. IU penicillin and 1mg streptomycin per ml). The prepared suspension was kept at -20°C before use. Embryonated chicken eggs were inoculated through allantoic cavity and chorioallantoallantoic membrane routes. Embryos were examined for specific P.M. lesions, CAM for plaques and allantoic fluid for HA activity.

Haemagglutination (HA) and Haemagglutination Inhibition (HI) Tests:

HA test was carried out after Anon (1971). HI test was carried out after Gough (1974).

Agar Gel Precipitation test (AGP):

AGP test was carried out after (Woernle 1963).

The pathogenicity of the isolated IB virus strain:

Five birds 10 days old were selected randomly, sacrified and postmortemly examined. Sera collected and tested for IB antibodies . One hundred and seventy-five, ten old chicks were divided into seventeen equal groups (1-17), each containing 10 birds. Each group of the first 16 groups was infected with one isolate from the sixteen IB isolates through both ocular and tracheal instillation route for each bird. Each bird received 0.2 ml with a titre ranged from 106.3 - 106.9 (table 4). Birds of group (17) were kept as a negative control. All experimental birds were clinically observed for 7 days post infection. Dead birds and survivors were subjected to postmortem, and serological examination and reisolation trials.

The pathogenicity of isolated Reovirus strains:

Five birds 10 days old were selected randomly, sacrified, P.M. examined and their sera collected and tested agaist reovirus antibodies. Eightly five 10 day old chicks were divided into eight equal groups (1-8), each containing 10 birds. Each group of the first seven groups was infected with one isolate from 7 reovirus isolates through both ocular instillation and foot bad inoculation routes. Each bird received 0.2ml. with a titer ranged from $10^{5.3}$-$10^{8.3}$ (table 4). Birds of group 8 were kept as a negative control. All experimental birds were clinically observed for 7 days post infection. Dead birds and survivors were subjected to postmortem and serological examination and reisolation trials.

The pathogenicity of the isolated ILT virus strains:

Fifty birds 42 days old chicks were divided into 4 equal groups (1-4) each group containing 10 birds. Each groups of the first 3 was infected with isolates, through both ocular and tracheal instillation routes for each bird. Each bird received 0.2 ml with a titer from $10^{5.7}$-$10^{5.75}$ table (4). Birds of group 4 were kept as a negative control. All experimental birds were clinically observed for 7 days post infection. Dead birds and survivors were subjected to postmortem, serological examination and reisolation trials.

The pathogenicity of the isolated Pox virus strains:

Five birds 35 days-old were selected randomly, sacrificied, P.M. examined, sera collected and tested against pox virus antibodies. Thirty, 35 day old chicks were divided into equal groups (1-30 each containing 10 birds. Each group of the first 2 groups was infected with one isolate from the 2 pox isolates using wing web and feather follicle methods for each bird. Each bird received 0.2ml. of a titer ranged from $10^{6.5}$-$10^{7.25}$ table (4). Birds of group 3 were kept as a negative control. All experimental birds were clinically observed for 7 days post infection. Dead birds and survivors were subjected to postmortem and serological examination and reisolation trials.

RESULTS

Virus Isolation:

Isolation trials were carried out using embryonat-

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ng chicken eggs. Results revealed the isolation of orty-one isolats.
esults are summarized in table (1)

dentification of isolated strains:

gar Gel precipitation test revealed that the 41 gents isolated were identified as 13 NDV (9.9%). 6 IB (11.6%), 3 ILT (2.2%), 2 pox virus (1.9%), nd 7 Reovirus (4.4%) (table 1).
arther tests were carried out for NDV identification including HA and HI test using ND antisera.

Heat stability of ND haemagglutinin:

Thirteen ND strains were tested for their thermostability at 56 C° for 15, 30,60,90 and 120 min. Results revealed that five isolates, were thermolabile at 30 min, one isolate at 60 min.,3 isolates at 90 min., and 2 isolates at 120 min. (table 2).

Serological investigation:

Sera collected from live clinical cases were tested for antibodies against ND virus. IB virus, ILT virus, Pox and reoviruses using HI and AGP test. Results are shown in table (3) from which it appeared that the percentage of antibody demonstration were as follows ND (9.9%), IB (11.6%), ILT (2.2%), pox (1.9%) and reovirus (4.4%).

Incidence of Different viral complicating Agents of clinical Coryza:

Virus isolation trials showed that NDV, IBV, ILT, Pox viruses and reovirus associated with clinical cases of coryza. moreover reovirus was mixed with ND and IB viruses in two cases.

The pathogenicity of IB virus isolates strains:

The inoculated 10 day old chicks showed depression, respiratory signs, diarrhoea, sneezing and rales. The mortality rats ranged from 10-30% (table 4). The postmortem findings were grayish-viscus mucus bluges observed in the Lumen of trachea and bronchi. The tracheal mucosa was congested and lungs revealed focal areas of hepatization and congestion. The air sacs were thickened and cloudy.

The pathogenicity of isolated Reovirus strains:
The inoculated 10 days chicks revealed depression, respiratory signs, swollen hock joint and lameness. The mortality rate was ranged from 0-30% (table 4). Postmortem changes were thickened tracheal wall with congested mucosa covered with transparent mucus. The lungs were slightly necrotic foci. Catarrhal enteritis and splenomegaly.

The pathogeniciy of isolated ND virus strain:

The inoculated 21 day old chicks revealed depression, respiratory signs, greenish diarrhea and ruffled feather table 4. Postmortem changes were congested lungs, petechial haemorrhages above the proventricular glands, enteritis with ulceration and congestion of submeningeal blood vessels of the brain.

The pathogenicity of isolated ILT virus strains:

The inoculated 42 day old chickens revealed rales , coughing, sneezing and loss of appetite. The mortality rate among chicken groups ranged from 0-20% (table 4). Postmortem changes were bloody exudate in larynx and tranchea and the lung were congested.

The pathogenicity of isolated Pox virus strains:

The inoculated 35 day old chickens revealed wart-like nodules on comb wattles and birds were ema-

Table (1): Virus isolation and identification

No. of cases with dinical coryza	No. of + ve cases	ND	IB	ILT	Pox	Ree
138	41	13 9.9 %	16 11.6 %	3 2.2 %	2 1.9 %	7 4.4 %

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Table (2): Heat stability of the ND haemagglutinin at 56 Co

Isolate	ND HA litre	HA titre at 56°C time/minute					Heat stability
		15	30	60	90	120	
1	1:640	1:10	--	--	--	--	30 min
3	1:2560	1:80	--	--	--	--	30 min
23	1:2560	1:160	--	--	--	--	30 min
27	1:1280	1:640	1:160	1:20	--	--	60 min
32	1:1280	1:160	1:160	1:20	--	--	60 min
38	1:2560	1:160	--	--	--	--	30 min
87	1:2560	1:640	1:320	1:160	1:40	1:20	120 min
91	1:1280	1:640	1:32	1:160	1:80	1:10	120 min
94	1:1280	1:320	1:160	1:180	1:20	--	90 min
101	1:2560	1:80	--	--	--	--	30 min
108	1:640	1:160	1:80	1:80	--	--	90 min
114	1:2560	1:640	1:320	1:80	1:40	--	90 min
130	1:320	1:80	1:20	--	--	--	30 min

Table (3): Serological examination

Antigen	ND	IB	ILT	Pox	Reo
Antibody %	7.3	10.4	1.5	0.73	9.9

Table (4): Pathogenicity of viral isolate inchicken.

Strain	Age/days	Route	IP/day	Mortality	Reisolation
IB 16 strain	10	ocular Traches	3-5	1-3/10	+ve
Reo 7 strain	10	ocular Trachea Foot bad	5	0-3/10	+ve
ND 13 strain	21	ocular Trachea	3-5	0-5/10	+ve
ILT 3 strain	42	ocular Trachea	6-8	0-2/10	+ve
Pox 2 strain	35	Wing web FF	7-8	0-1/10	+ve

ciated.

DISCUSSION

In this study 138 specimes repressenting 138 flocks suffering from clinical coryza were examined for the presence of complicating or associated viral agents. Serological testing of sera collected from the affected flocks revealed the presence of precipitating antibodies for ND virus (7.350. IB virus (10.4%). Reovirus (9.9%), ILT virus (1.5%) and Pox virus (0.73%). Thirteen haemagglutinating agents were isolated in embryonated chicken eggs. haemagglutination activity of these isolates were carried out and their titer ranged from 1:60 to 1:2560.

Haemagglutination inhibition carried out with 4 HA units of known specific ND antiserum, revealed that the 13 isolates NDV. Similar results

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were also recorded by Andrews and Pereira (1972), Hunson and Spalatin (1978). The pathogenicity of NDV isolates for 3 weeks-old chickens revealed that the incubation period in experimentally infected birds was 2 to 7 days. These results indicate that the viruses are of different pathotypes and intravenous pathogenic index or intramuscular pathogenic index should be determined (Atiat and Nasr 1964, Parede and Young 1989).

Experimentally infected birds with the isolated NDV showed respiratory signs and gross lesions, these changes were similar to these observed by Hanson 1972, Hanson (1975), and Lancaster (1981). On the other hand 16 isolates were identified as infectious bronchitis virus, isolated on allantoic cavity of embryonated chicken eggs. The pathogenicity of IB virus isolates to 10 day-old chickens revealed that the incubation period was 4 days. The observed signs and gross lesion were mainly localized in respiratory system, sneezing, rales, depression and rhinitis. These results were similar to these previously described by Gelb et al. (1991).

In this study, three viruses identified as ILT, were isolated on the chorioallantoic membrane of embryonated chicken eggs. The pathogenicity of ILT virus isolates to 42 day-old chicks revealed that the incubation period in infected birds was 7 days. The observed signs and gross lesions were similar to these previously described by Jenifier et al. (1991). In addition, two virus isolates identified as avian pox virus by AGP test using chorioallantoic membrane of embryonated chicken eggs. The pathogenicity of the two pox viruses for 35 day-old chickens revealed that the incubation period was 7 days. The observed clinical signs and gross lesions were similar to the previously described by Docherty et al., (1991). Furthermore in this study, seven virus isolates were identified as reovirus in mixed infection with IB virus and ND virus, isolated in the allantoic cavity and chorioallantoic membrane of embryonated chicken eggs . The pathogenicity of the seven reovirus to 10 day old chickens revealed that the incubation period in infected birds was 5 days. The observed signs and gross lesions were also observed by Hill et al., (1989).

From the present study it is observed that mixed infection by viruses were observed in chicken flocks suffering from coryza troubles ND virus,IB, ILT virus, Pox virus and Reovirus, Moreover, reovirus was mixed with ND and IB viruses in two cases.

Finally, it could be concluded that the NDV, IBV, ILT virus and reovirus were associated with the infectious coryza whenever hemophilus was isolated or not in chickens in a separated or mixed forms. good hygienic and sanitary measures are recommended for poultry farms beside good vaccines necessary prevent the virus infections so to decrease the respiratory troubles specially in broilers.

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