VIRAL AGENT ASSOCIATED WITH ARTHRITIS IN QUAIL IN KALYOUBIA AND MINUFIYA PROVINCES OF EGYPT

By

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SUMMARY

An outbreak of arthritis was reported in two private quail farms for meat and/or egg production in kalyoubia and Menoufiya Provinces. Fifty adult arthritic Japanese quails were collected from these farms and examined clinically and subjected to viral examination. Out of the 50 arthritic adult quails, nineteen joints (38%), eleven lungs (22%) and nine livers (18%) were positive for reovirus isolation.

Groups of free and susceptible one-week-old quails and one-day-old broiler chicks were used to test the pathogenicity of the recovered reovirus by oral, subcutaneous (s\c) and intrajoint (i\j) inoculations.

The inoculated reovirus produced mortality, arthritis and decrease in body weight in both inoculated quail and chicks.

INTRODUCTION

Avian reoviruses are among the causes of viral arthritis and tenosynovitis in chickens (VanDer Heide 1977; Kibenge and Wilcox 1983; Robertson and Wilcox 1986 and Rosenberger and Olson 1986). Reoviruses have also been associated with a disease condition characterized primarily by enteric disease signs, as, diarrhea, poor feathering, poor feed conversion and stunting (Robertson and Wilcox 1986). This disease condition has been given several names, including malabsorption syndrome (Robertson and Wilcox 1986 and Clark et al., 1990). In addition, several other disease conditions such as pericarditis /myocarditis and hepatitis, have been reported in avian reovirus infected chickens (VanDer Heide 1977 and Robertson and Wilcox 1986).

Avian reovirus infections have also been recognized in turkeys (Page et al., 1982).

For the poultry industry, avian reoviruses represent important pathogens, since they produce mortality, leg weakness, and poor feed conversion as well as depressed productivity (Rosenberger and Olson 1986).

Natural avian reovirus infection occurs mainly via the oral- fecal route (Sahu and Olson 1975 as well as Jones and Onunkwo 1978). Viral entry and initial replication occurs in the intestine and bursa, followed by viral spread via blood to other tissues or organs (Kibenge et al., 1985 and Jones et al., 1989). Viruses have been detected in many tissues of infected chickens, including the intestine, bursa, lung, liver, spleen, heart and hock joint (Menendez et al., 1975; and Robertson and Wilcox 1986).

Commercial production of game birds such as bobwhite quails has increased in recent years. These advances are due partly to the adoption by quail producers of husbandry practices of the commercial poultry industry such as growing birds in high densities in inclosed environments (Magee et al., 1993). In year 2000 cases of arthritis appeared among quails in two private farms in kalyoubia and Menoufiya Provinces. Saad et al., 2001 were successful in isolating several bacterial agents, and this study was planned to determine the role of viruses in etiology of arthritis in quails. Therefore a trial for isolation and identification of incriminated viruses in causing arthritis in quails was made and their role to reproduce arthritis in susceptible baby quails and chicks was studied. In addition the effect of viruses on body weight of experimentally inoculated birds was evaluated.

MATERIAL AND METHODS

1- Samples:

Lungs, livers and hock joints of the 50 arthritic diseased and dying quails were collected from two private farms for meat and/or egg production in kalyoubia and Menoufiya Provinces.

Small parts from lungs, livers and tendon sheaths and/or synovial fluids of 50 arthritic quail were ground and 10% suspensions were made using physiological buffer saline (PBS) containing antibiotics (10,000 i.u penicillin, 1000 mg streptomycin and 250 ug amphotericin/ml). The suspension was left for one hour at-4°C, homogenized, centrifuged and supernatant stored at -20°C till used for virus isolation.

Embryonatd chicken eggs (ECE)

Ten day-old ECE obtained from El-Fayoum. Kom-Oshim. SPF Project. Ministry of Agriculture. Egypt. were used for virus isolations, propagation, and re-isolation trials from experimental birds as well as the serum neutralization test.

Virus and antisera

A reference reovirus, S-1133 and chicken immune sera against reovirus S-1133 supplied from Department of virology Animal Health Research Institute (Dokki), were used in the serological tests through out this investigation.

Virus isolation and Identification

It was accomplished by inoculating 0.1ml of sample supernatant into 10-day-old (ECE) on to chorioallantoic membrane (CAM). Embryonated eggs were candled twice daily for 8 days. Embryo mortalities and lesions were recorded. All deaths occurring within 1st 24 hours postinoculation were regarded as non specific. From

embryos dying between 1-8 days postinoculation, CAMs were harvested aseptically and stored at -20°C. At least three blind passages were made before a sample was considered negative.

Haemagglutination test (HA)

Allantoic fluid of all virus isolates were tested for HA activity by rapid slide and slow plate HA tests according to (Anon 1971) using 1 and 0.75% washed chicken erythrocytes, respectively.

Chloroform sensitivity

Sensitivity of virus isolates to chloroform was done according to Lukert 1989.

Agar gel precipitating test (AGPT)

Virus identification was performed by testing affected CAM material in an Agar gel precipitin test (AGP) against reovirus (Vaccine strain S1133) and antiserum (Bulow and Biggs, 1975).

Serum neutralization test (SN)

The fixed serum- variable virus method was used according to the technique described by (Beard, 1980) and who carried out on the CAM of embryonated chicken eggs.

Experimental infection in one-week old quail

Twenty eight one-week old Japanese quail were pre-tested for freedom from reovirus antibodies by (AGPT), were divided in to four equal groups. First group was left as non inoculated control, second, third and fourth groups were respectively inoculated per os, s/c and i/j with 0.05ml of a 10^{-2} dilution of the virus made in phosphate-buffered saline (PBS) after (Magee et al 1993).

Experimental infection in one –day- old chicks

Twenty eight one- day-old Hubbard chicks were purchased from private hatchery and pre-tested for freedom from reovirus antibodies by AGPT. Chicks were equally divided into four groups. The first group was kept as non inoculated control group, while the 2nd , 3rd, and 4th group were respectively inoculated per os, s/c and i/j with 0.5 ml ($10^{5.75}ELD_{50}$) reovirus/ chick after (El-Zanaty 1993).

Inoculated and control birds (quails and chicks) were housed separately in isolated units and observed daily for morbidity and mortality. Dead quails or chicks were removed and frozen. The surviving quails and chicks in each group were weighted weekly and the mean body weight of each group was recorded. At the end of the experiment hock joints, livers and lungs from each dead or surviving quail or chick were collected, processed and inoculated into ECE for re-isolation of inoculated virus.

The statistical analyses of the data were made according to CoStat program (1986).

RESULTS AND DISCUSSION

In this study, we tried to explore the possible role of viruses in causing arthritis in quails and this was undertaken after our first trial of isolation the incriminated bacteria in producing arthritis in the same aforementioned two flocks and same samples of 50 arthritic quail (Saad et al., 2001).

The naturally affected quails showed ruffled feathers, off food, lameness, hot and swollen joints especially hock joints. Others represented bumble foot. In addition to high mortality in the two donor flocks. Necropsy findings showed swollen joints containing exudates, congestion of internal organs, livers enlargement and in some cases small necrotic foci. In addition to congestion of duodenal mucosa were observed, catarrhal enteritis was a common findings. Our results are similar to some extent with results of (Rafique et al., 1996) who recorded 7.09% mortality in broilers naturally infected with reovirus. The lesions were necrosis of femur head, acute to chronic enteritis and enlargement of liver and spleen with few necrotic foci on liver.

Viral examination of 50 arthritic adult quails collected from two private quail farms for meat and/or egg production from 2 Provinces by using HA, chloroform sensitivity, AGPT and S.N tests, revealed that 39 (78%) of them were positive for reovirus isolation as shown in Table (1). Incidence of viral isolation from different organs examined is indicated in Table (2). From the table it is obvious that 19 (38%) joints, 11 (22%) lungs and 9 (18%) livers were positive for reovirus isolation.

The presence of reovirus in quail is supported by (Ritter et al., 1986) who isolated reovirus and cryptospordium sp from Bob white quail with enteritis. Also (Magee et al., 1993) recorded high mortality in young Bob white quail due to reovirus infection.

To our knowledge no data about arthritis/tenosaynovitis in quail due to viral causes were recorded. As all available literatures did not deal with viral or bacterial arthritis/ tenosynovitis in quails. On other hand several reports have been published on arthritis/tenosynovitis in chickens due to reovirus infection (Sharifah et al., 1989; Rafique et al., 1996 and shirakawa et al., 1997) who could isolate avian reovirus from different flocks of broiler with tenosynovitis.

The pathogenicity of our isolated reovirus was tested against both one-week old Japanese quail and one-day-old- broiler chicks through different routes.

One-week old Japanese quails tested for freedom of antibodis to reovirus, by (AGPT or S.N test) were inoculated with 0,05 ml of 10^{-2} dilution of the virus made in PBS, through per os (group 2), s/c (group 3) and i/j (group 4). The degree of pathgenicity as judged by mortalities are illustrated in table (3). The inoculated quails (per os- group 2) showed depression, tendency to sit, ruffled feathers, lameness, diarrhea and mortality at a rate of 2/7 (28.5%) two hock joints from 7 inoculated quail (28.5%) appeared hot, immbolized and swollen, as seen in table (3). Subcutaneous inoculation of one-week old Japanese quails (group 3) with 0.05 ml of 10^{-2} dilution of virus made in PBS, yielded clinical signs to some degree similar to group 2 except high mortality 5/7 (71.42%) compared to group 2 and group 1 was recorded as seen in table (3). Intrajoint inoculation of one-week old Japanese quail with 0.05 ml of 10^{-2} dilution of virus in PBS (group 4), showed less severe clinical signs compared to group 2 and group 3 respectively and low mortality 1/7 (14.28%),

was record while the number of affected joint 4/7 (57.14%) surpassed that of group 2 and group 3 (2/7) for each.

Results of pathogenicity against reovirus in one-week old quails through different routes were to some extent similar to those of Magee et al., 1993 who stated that, in experimental trial, which used the reovirus isolated from excessive mortality in young Bob white quail, produced both mortality and the morbidity characteristics and lack of vitality, and Minta et al., 2001 who record 60-80% mortalities in broilers inoculated orally by reovirus. While Guy et al., 1987 noticed that quails inoculated with reovirus alone did not develop clinical apparent diseases and the infection was localized principally in the intestinal tract and no lesions were detected.

Necropsy of inoculated quails revealed pale livers, catarrhal enteritis, enlarged proventriculus, swollen and oedematous foot pad and hock joint. These results to some extent are similar to that of donor flocks.

Body weight of the three inoculated groups become lowered when compared to control non inoculated group as seen in Table (5). These findings are in agreement with those published by Magee et al., 1993. On other hand Guy et al., 1987 stated that mortality and weight gain of quails infected with the reovirus alone was similar to those of control non inoculated group which disagreed with our data.

Reovirus was reisolated from all quail that died during experiment (two from group 2, five from group 3 and one form group 4). At the end of the experiment the remaining quails (7 control, 5 inoculated by oral route, 2 inoculated by s/c route and 6 inoculated by i/j route) were bled and necropsied, reovirus was recovered from two of group 2 (lung and joint), one from group 3 (lung and joint) and two from group 4 (joint only). No reoviruses were recovered from the control quails (group 1) as seen in table (3). These results partially agreed with results of Guy et al., 1987 who isolated reovirus only once, on day 6 post inoculation from visceral organs of quails inoculated s/c with reovirus only.

The pathogenicity of isolated reovirus from quails was tested in one-day-old broiler chicks by inoculation of 0.5ml ($10^{5.75}$ ELD_{50}). Reovirus through per os, s/c and i/j inoculation routes.

The inoculated chicks showed diarrhea which began to appear from 3rd day postinoculation. Most of inoculated chicks had distended abdomen (15-21 days postinoculation) except that chicks of group 4 (intrajoint inoculation), growth impairment was noticed by one week post inoculation, thus body weight was less than of control group (non inoculated group) as seen in Table (6), mortalities were recorded in Table (4). Our results are similar to that of Shira et al., 1990 and El-Zanaty., 1993.

Post-mortem findings revealed enlargment of proventriculus decrease in the gizzard size, decrease in size of spleen and pancreas, these post mortem lesions were obvious in groups 2 and 3 but not found in group 1, (control non inoculated) and to lesser extent- in group 4 (intrajoint inoculated group). These findings agree with those reported by Shirai et al., (1990); El-zanaty (1993) and Montgomery et al., (1997). Arthritis and mortalities in inoculated chicks are summarized in (table 4). Our results of clinical signs and post-mortem findings which appeared on chicks

inoculated with reovirus are similar to some extent to stunting syndrome which usually appear in broiler chicks after infection with reovirus and/or other pathogens. These results are in general agreement with the findings of. Rekik et al., 1991 who recorded that reoviruses are in association with pathological conditions other than viral arthritis.

Concerning chicks of group 4, some of the inoculated birds (two chicks) showed swollen joints and foot pad with exudate and developed inflammed tendons. These results are similar to those of Sharifah et al., 1989. Reovirus was recoverd from all died chicks and from some of survivors which bled after the end of the experiment as seen in Table (4) .

It could be concluded from both recorded results of Saad et al., 2001 and the obtained results in this paper that the incriminated bacteria and viruses in producing arthritic cases in two flocks of adult quail (in Kalyoubia and Menoufiya Provinces) are Mycoplasma synoviae, Salmonella virginia, Salmonella rubislaw and Salmonella haardt, in addition to reovirus. Since, we did not include one general table including the presence of virus isolates with or without each of the previously isolated bacteria by Saad et al., 2001 we can not include or exclude the possible synergestic or additive effects of the isolates. However it is clear that the isolated reovirus are highly pathogenic to quails and baby chicks.

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Table (1) locality and incidence of virus isolation from examined quail

Locality	No of examined quail	Positive virus isolation	%
Kalyoubia	39	31	79.48
Menoufiya	11	8	72.72
Total	50	39	78

Table (2) Incidence of reovirus recovery from examined organs.

Species	No. 1	reovirus recovery					
	Examined sample	Organ	%	Organ	%	Organ	%
		joint		Lung		livers	
Quail	50	19	38	11	22	9	18

Table (3) experimental infection of one-week old quail.

Group number	virus	Route of inoculation	No of inoculated quail	No of arthritic quail	% (a)	Deaths			Re-isolation Of the inoculated virus	
						No	% (b)	Days post infection	No.	% (c)
1	Non	Control	7	0	0	0	0	0	0	0
2	Reov irus	Per os	7	2	28.5	2	28.5	3-10	4	57.14
3	Reov irus	s/c	7	2	28.5	5	71.42	3-13	6	85.71
4	Reov irus	Intra joint (i.j)	7	4	57.14	1	14.28	5th day post inoculation	3	42.85

(i) = number of affected joint over number of inoculated × 100

(ii) = number of dead quails over number of inoculated × 100

(iii) = number of +ve re-isolation over samples tested of inoculated × 100

Table (4) experimental infection in one-day-old chicks.

Group number.	Inoculated virus	Route of inoculation	No. of inoculated chicks	No. of arithritic chicks	% (a)	Deaths			Re-isolation Of the inoculated virus	
						No.	% (b)	Days post infection	No	% (c)
1	Non	-	7	0	0	0	0		0	0
2	reovirus	Peros	7	0	0	1	14.28	On day 13 post inoculation	2	28.57
3	reovirus	s/c	7	0	0	3	42.85	3-11	4	57.14
4	reovirus	i.j	7	2	28.5	1	14.28	On day 17 post inoculation	1	14.28

(i) = number of affected joint over number of inoculated × 100

(ii) = number of dead chicks over number of inoculated × 100

(iii) = number of +ve re-isolation over samples tested of inoculated × 100

Table (5) Effect of reovirus on body weight of one-week old quails (changes mean in grams)

Age/ week	Group 1	Group 2	Group 3	Group 4	Ms	
					Error	Treat
1st week (5-day-old)	a 16.58±0.24	d 16.35±0.13	C 16.51±0.13	b 16.96±0.1	0.026	0.474
2nd week	a 25.41±0.09	d 21.84±0.07	C 22.34±0.06	b 22.85±0.09	0.006	17.78 **
3rd week	a 73.71±0.49	d 63.28±0.08	C 65.57±0.07	b 66.71±0.12	0.067	141.566 **
4th week	a 166.57±0.13	d 149.42±0.13	C 154.17±0.09	b 160.71±0.13	0.015	393.79 **

a, b. means in the same row with different superscripts differ significantly (P<0.05)

** = P < 0.01

Table (6) Effect of reovirus on body weight of one-day-old chicks (changes mean in grams)

Age/week	Group 1	Group 2	Group 3	Group 4	Ms	
					Error	Treat
1st week	53.24±1.59	51.48±2.39	53.06±3.76	51.17±3.55	8.77	7.89
2nd week	a 162.5±24.34	b 117.8±15.14	b 134.4±21.6	ab 141.6±101.8	496.91	2401.05 **
3rd week	a 465.11±21.11	c 391.47±12.88	c 401.67±14.3	b 421.71±21.6	318.88	7438.79 **
4th week	a 664.2±28.72	c 591.67±29.68	bc 602.82±21.09	b 622.71±21.48	633.22	7134.95 **

a, b, means in the same row with different superscripts differ significantly (P< 0.05)
** = P<0.01