



Immunological and molecular methods for detection and evaluation mumps outbreak among children in Baghdad city

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Abstract

Mumps is an acute infectious disease, caused by a paramyxovirus, it is related strictly to para-influenza virus. Generally, it is a mild disease but occasionally has severe complications. Mumps tends to occur in high altitudes in some Iraq regions. The seasonality of mumps is now less manifest, and suitcases may happen year-round. Outbreaks and cases may arise sporadically, and infected individuals who were exposed to mumps cases or likewise who traveled to mumps endemic regions outside Iraq recently. It has been improved from saliva, milk, urine, cerebrospinal fluid, blood, and other infected tissues. Promptly it is inactivated by formalin, ether, heat, UV, and chloroform. Generally, the disease is mild cases, but approximately 10% of patients can grow aseptic meningitis, and a less common complication to encephalitis, which can lead to disability or death, pancreatitis, orchitis, permanent deafness, and other unfortunate effects. Humans are considered the lone natural host for mumps virus, which is spread by respiratory droplets typically. The incubated period for this virus is (16-18 days), with a diversity of about 2-4 weeks.

Keywords: Mumps, effective factors, Iraqi children, ELISA, PCR method, Baghdad city

الطرق المناعية والجزيئية للكشف وتقييم تفشي النكاف بين الأطفال في مدينة بغداد.
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المستخلص

النكاف هو مرض معدٍ حاد تسببه فيروسات الباراميكسو، وهو مرتبط ارتباطاً وثيقاً بفيروس نظير الإنفلونزا. يُعد النكاف عمومًا مرضًا خفيفًا، لكنه قد يؤدي أحيانًا إلى مضاعفات خطيرة. ينتشر النكاف في بعض مناطق العراق ذات الارتفاعات العالية. لم تعد موسمية النكاف واضحة كما في السابق، حيث يمكن أن تحدث الحالات على مدار العام. قد تظهر تفشيات وحالات متفرقة، حيث يمكن أن يُصاب الأفراد الذين تعرضوا لحالات نكاف أو الذين سافروا مؤخرًا إلى مناطق موبوءة بالنكاف خارج العراق. يمكن عزل الفيروس من اللعاب، الحليب، البول، السائل الدماغي النخاعي، الدم، والأنسجة المصابة الأخرى. يتم تعطيل الفيروس بسرعة عند تعرضه للغورمالين، الإيثير، الحرارة، الأشعة فوق البنفسجية، والكلوروفورم. عادةً ما يكون المرض خفيفًا، ولكن حوالي 10٪ من المرضى قد يُصابون بالتهاب السحايا العقيم، كما أن أحد المضاعفات النادرة هو التهاب الدماغ، الذي قد يؤدي إلى الإعاقة أو الوفاة، بالإضافة إلى التهاب البنكرياس، التهاب الخصيتين، فقدان السمع الدائم، ومضاعفات أخرى خطيرة. يُعتبر البشر المضيف الطبيعي الوحيد لفيروس النكاف، الذي ينتقل عادةً عبر الرذاذ التنفسي. تتراوح فترة حضانة الفيروس بين 16-18 يومًا، مع نطاق زمني يمتد من 2 إلى 4 أسابيع.

الكلمات المفتاحية: النكاف، العوامل المؤثرة، الأطفال العراقيون، اختبار ELISA، طريقة PCR، مدينة بغداد

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معلومات البحث

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Introduction

Mumps is known acute infection illness, it is a paramyxovirus in the same group as Newcastle and Parainfluenza viral disease. This viral disease

created antibodies that cross-reacted with the Mumps virus. This virus has a single-stranded RNA genome and can be isolated from

several cultures of human, and monkey tissues, and in embryonated eggs [1]. It has been improved from saliva, milk, urine, cerebrospinal fluid, blood, and other infected tissues. Promptly it is inactivated by formalin, ether, heat, UV, and chloroform [2]. Generally, the disease is mild cases, but approximately 10% of patients can grow aseptic meningitis, and a less common complication to encephalitis, which can lead to disability or death, pancreatitis, orchitis, permanent deafness, and other unfortunate effects. Although vaccination is the best approach to preventing mumps infection, it is not effective 100%, so the earlier vaccinated individuals can still catch mumps, and outbreaks have occurred in highly vaccinated individuals [3]. Humans are considered the lone natural host for mumps virus, which is spread by respiratory droplets typically. The incubated period for this virus is (16-18 days), with a diversity of about 2-4 weeks [4]. The most common symptoms included low-grade fever, headache, malaise, and anorexia. On the other hand, about one-third of cases of Mumps viral infection have been asymptomatic. The disease can differ from a mild upper respiratory infection to viremia, with extensive systemic involvement. Classic mumps is characterized by the parotid gland enlargement and other salivary glands [5]. The Mumps vaccine is available, joined with the measles, and rubella vaccines (MMR), or (Pro Quad) collectively with the measles, rubella, and varicella vaccine (MMRV). The Consultative Committee on Immunization Practices comments that the MMR vaccine be used when any of the population components is indicated [6]. In one huge study, the middle age for mumps orchitis was 29 years (range from 11-64 years), likewise, orchitis happens in men with about 25% of post-pubertal contract

mumps [7]. Generally among Mumps immunity, the individuals with a serologic indication of Mumps immunity have documentation of physician-diagnosed Mumps or may have records of vaccination with at least one dose of live Mumps vaccine after their first birthday [8]. The demonstration of IgG Mumps antibody by any common serological test is acceptable evidence of mumps immunity. The individuals who have an (equivocal) serological assay results, and should be reflected as susceptible to mumps immunity [9]. On the other hand, individuals who have a confusing serological test result should be considered vulnerable to mumps infection, and must to confirm by PCR technique for building the right strategy of disease control [8].

2. Materials and Methods

2.1 Patients

The number of stated mumps cases has progressively increased, from (112) cases to a total (210) cases Since January 2019. This reappearance predominantly affected children, with the maximum attack rates arising among those children living in remote regions of Baghdad, The mumps slightly outbreak arose from December 2019 to December 2022, conducted in Al-Yarmouk Hospital and teaching laboratories in Medicine City.

2.1.1 Laboratory Diagnosis

Mumps diagnosis is largely dependent on the presence of specific clinical pictures through diagnosis in clinics and hospitals, specifically the parotitis prevalence. This virus can be isolated from different clinical specimens. The swab

sample from the parotid duct, or the duct of another affected salivary gland. Mumps virus can also be diagnosed by polymerase chain reaction technique (PCR). Serology was the simplest technique for checking Mumps virus infection, EIA or ELISA is the most commonly used test. EIA is commonly available and is more sensitive than another serologic tests, it is presented for both IgM and IgG [10].

2.2 Methods

2.2.1 Clinical Diagnosis and Questionnaire

A clinical diagnosis of mumps was through on the symptoms basis of fever and constitutional symptoms like (Parotitis) evolving within 3 weeks after strong exposure. Salivary glands swelling, headache, fever, vomiting, and nuchal rigidity, appeared 3 to 5 days after the beginning of parotitis with a range of 1 week before and 2 weeks later. The study included the personality factors questionnaire about living status, number of family members, school status, previous diseases, prior infection with mumps, and vaccination system, as well as health care and hygiene [11].

2.2.2 The salivary test

The salivary test was designed to measure the antibodies:

- 1- IgM present was a validation of recent infection
- 2- IgG signified previous infection or immunization

The consequences of this were as follows

- 1- Detection of the antibodies measured after a week of onset of symptoms.
- 2- The salivary samples had been taken at the initial consultation.
- 3- Results were not available until the patient had fully recovered.
- 4- The samples for this test had been taken at the time of onset of symptoms.

2.2.3 Procedure for taking a salivary sample

The sample is taken by quietly sweeping the gums and teeth for a minute or two - with a sponge swab and is therefore unproblematic even in young children. This can be done by the clinician, or the parent [12].

2.3 Method

2.3.1 Kits of diagnosis

Mumps was measured by the method of Enzyme-Linked Immunosorbent Assay (ELISA) Human Anti-Mumps virus IgG ELISA Kit (ab108752) [13]. Then molecular method made by step Real-time PCR, of Zena Max-Mumps Virus (MuV) PCR Detection Kit CE-IVD, no. KD919169-100 from (Advanced Molecular Diagnostics Biocity Nottingham Pennyfoot Street, Nottingham, NG1 1GF, United Kingdom). [12].

2.3.2 ELISA method

The well plates were pre-coated with Mumps virus Ag to bind with cognate Ab, then control or test samples to wells and incubated. Followed with washing, then added HRP labeled anti-human IgG conjugate to the wells and bound to the immobilized Mumps virus-specific ab. TMB was then catalyzed by the HRP enzyme, and a blue

color was produced that changed to a yellow color after adding an acidic stop solution. The optical density of the yellow color was directly proportional to the Mumps virus IgG sample captured in the plate [13].

2.3.3 Real-Time Method:

The real-time detection method was based on the fluorogenic 5 nuclease test. During the polymerase chain reaction method, the probe cleaved at 5 ends by DNA polymerase and separated the reported dye from the quencher dye only when the probe hybridizes with the target DNA. The cleavage leads to a generated fluorescent signal, then monitored in real-time by a PCR detection system. The PCR elevated in the fluorescent signal, was detected initially (Ct), which was appropriate to the PCR product amount. After this monitoring, the fluorescent intensities were done in Real Time allowing the detection of the accumulation products, without having to re-open the tube reaction after the amplification [10].

2.3.4 Statistical analysis

Data have been statistically analyzed the usage of the SPSS program (model 18 and statistically, the facts were evaluated via using Microsoft Program, with percentages and charts [14].

3. Results

3.1 Mumps clinical disease features

Mumps outbreak has occurred in cases, was in about (3-12) year old infected, where approximately (115) 54.8 % reports of laboratory-confirmed mumps were received by many hospitals in Baghdad, from a total of (210) related cases were reported, was also confined with about (95) 45.2 % of cases occurring among children outside Baghdad community. The largest number and percentage of cases (57) 50% occurred among children aged 3–7 years, in Baghdad and (46) 48% outside Baghdad. Most of the patients were in 52 males (90), while females in 51 (120). as shown in Figure (1) and figure (2) ,Table (1).

Table 1: Patients infected by age, vaccinated, gender, regions

Patient no. = 210		Age	vaccinated		Gender				Region
No.	%	age	No.	%	Male	%	Female	%	
115	54.8	3–7 years	66	57	48	42	67	58	Baghdad
95	45.2	8–12 years	52	55	42	44	53	56	Outside Baghdad
Total	100		118	56%	90	43%	120	57%	

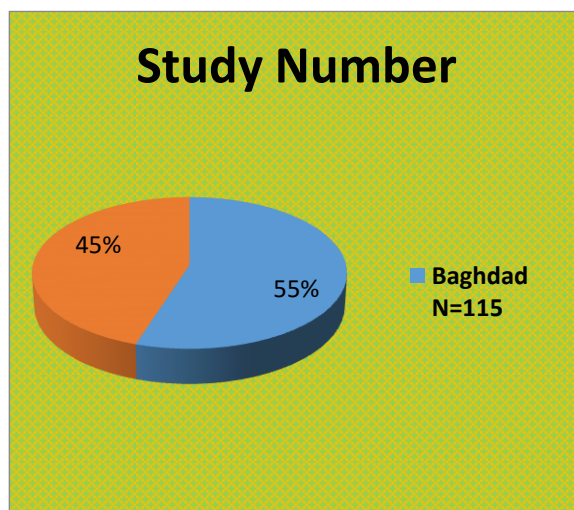


Figure (1): Patients infected Number

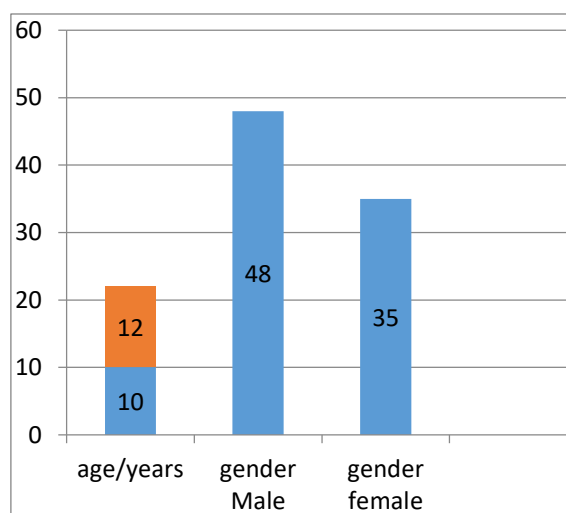


Figure (2): Patients by Age and Gender

The largest number and percentage of cases (57) 54.8 % occurred among children aged 3–7 years, in Baghdad and (46) 45.2 % in outside Baghdad.

Most of the patients were males 52, with female were 51. as shown in Table (2) Figure (3) and figure (4).

Table (2): Past infected Patients and Vaccinated in Baghdad region and outside

Patient no. = 210		Age	Past infected		by Gender				Healthy control no. = 30
No.	%		No.	%	Male	%	Female	%	
Bagdad (115)	54.8	3–7 years	57	50	30	26	27	23	12

Outside (95)	45.2	8–12 years	46	48	22	23	24	25	18
Total	100		103	98	52	50	51	50	30
vaccinated			62	60	36	58	26	42	28
Non vaccinated			41	40	16	39	25	61	2

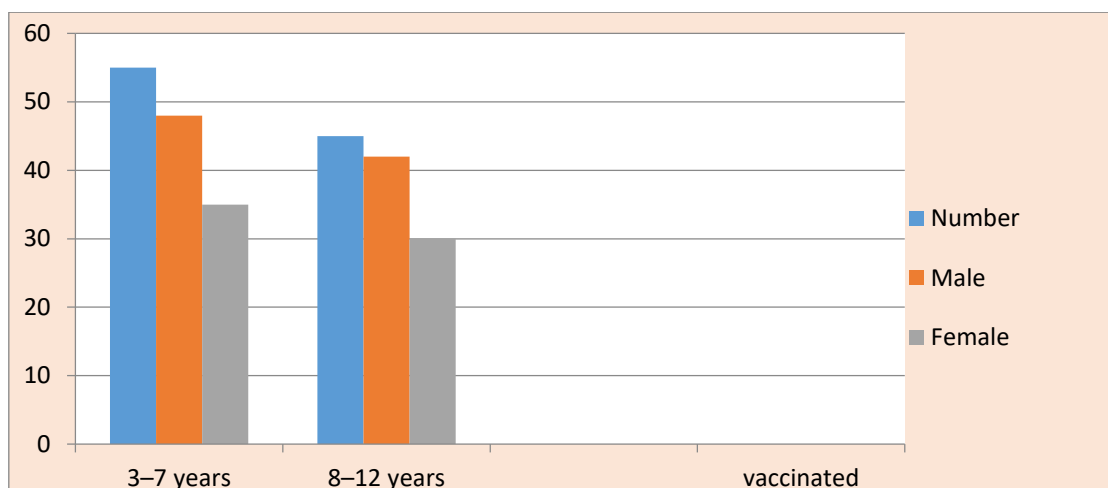


Figure (3): Patient with Past infected by Gender

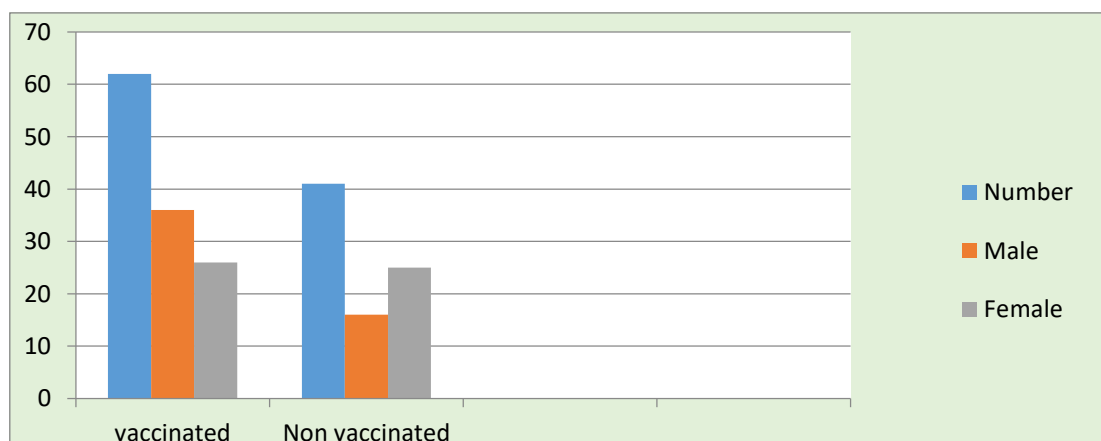


Figure (4): Past infected with vaccinated and non-vaccinated Patients by Gender

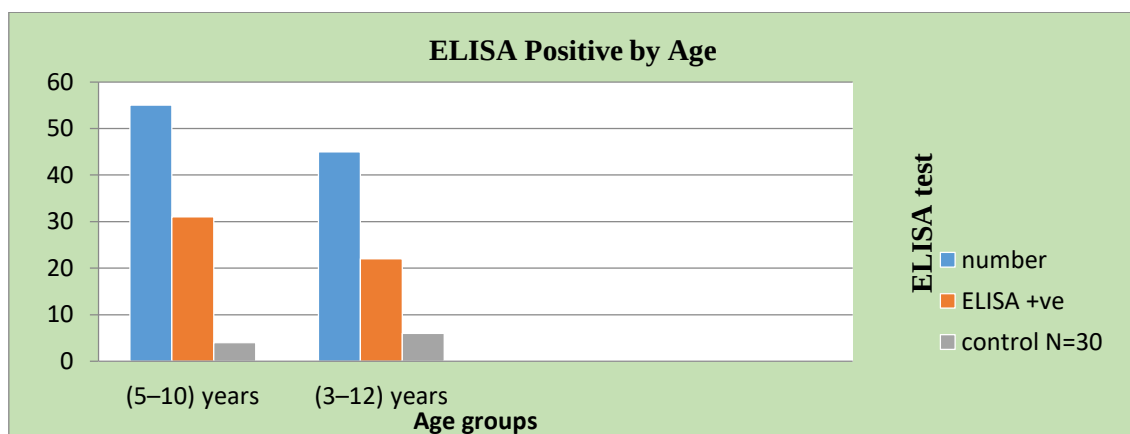
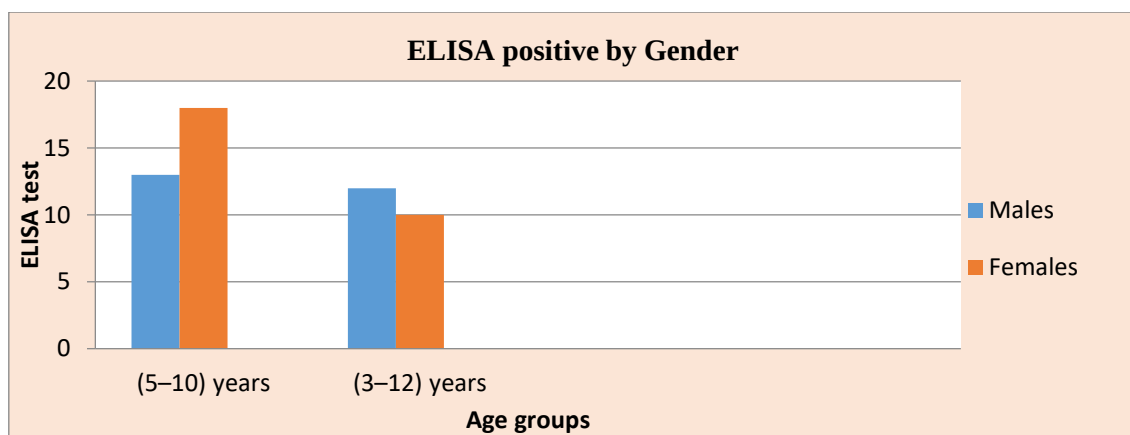
3.1.2 Results by IgG +ve by ILISA test

The result appeared by IgG +ve (31) were (male 13, Female 18) within age group (6–12 years), and (22) patients within age group ((3–5) years) were

as (12 males and 10 females), with control of (4, 6) within age groups respectively. as shown in Table (3) Figure (5) and figure(6).

Table (3): Patient in Baghdad region by IgG +ve by ILISA test

Total Patient no. = 210		Most infected Age		Gender		Healthy control N= 30		
Baghdad Pati. No.	%	Age groups	No.	IgG+ve	Male	Femal e	No.	+ve
115	54.8	(6–12) years	55	31	13	18	12	4
		(3–5) years	45	22	12	10	18	6
Total	100		100	53	25	28	30	10

**Figure (5): ELISA Positive by Age in Study and Control****Figure (6): ELISA Positive by Age and Gender in Study****3.1.3 Results with IgM +ve by ILISA test**

The results were showed that (115) total persons infected with mumps in Baghdad, Mumps occurred in (55) patients and the result of IgM+ve

were (31) patients, in age group (5–10) years, with control of (4, 6) within age groups respectively. as shown in Table (4) .

Table (4): Patient in Baghdad region with IgM +ve by ILISA test

Total Patient no.	Most infected Age	Gender	Healthy
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= 210							control N= 30	
Baghdad Pati. No.	%	Age groups	No.	IgM +ve	Male	Female	No.	+ve
115	54.8	(5–10) years	55	31	13	18	12	4
		(3–12) years	45	22	12	10	18	6
Total	100		100	53	25	28	30	10

3.1.4 Results by RT-PCR results

The result appeared by PCR method firmed most positive result with patients, mostly was within age group (3-5) years was (35), mostly within male gender was (18) and female (17), while in age

group (3-5) years was within male (14), and female (11). So results mostly were among male gender children in both groups. as shown in Table (5) ,Figure (7) and figure(8).

Table (5): Patient in Baghdad region by RT-PCR results

Total Patient no. = 210			Most infected Age		Gender		Healthy control N= 30	
Baghdad Pati. No.	%	Age groups	No.	+ve	Male	Femal e	No.	+ve
115	54.8	(5–10) years	55	35	18	17	12	1
		(3–5) years	45	25	14	11	18	2
Total	100		100	60	32	28	30	3

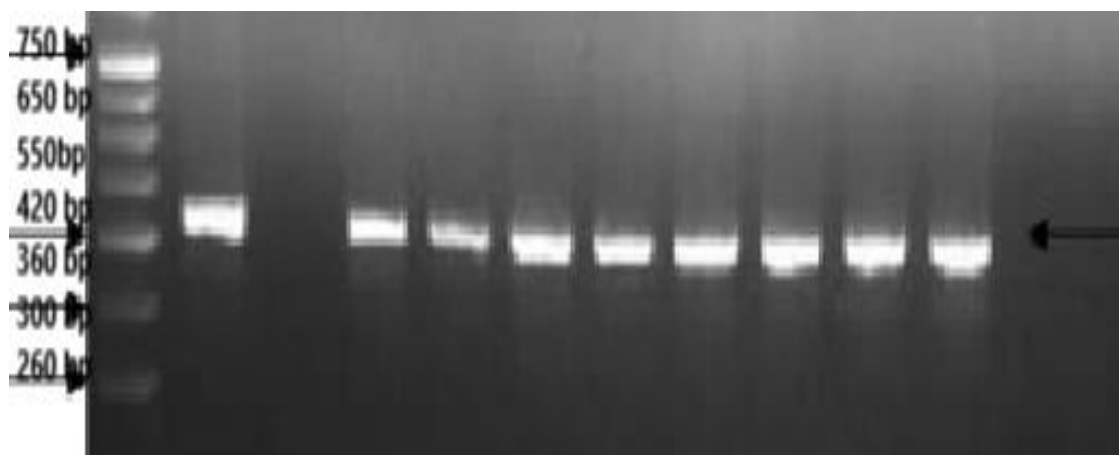


Figure (7): Agarose electrophoresis for mump RNA by PCR showed some positive results of samples

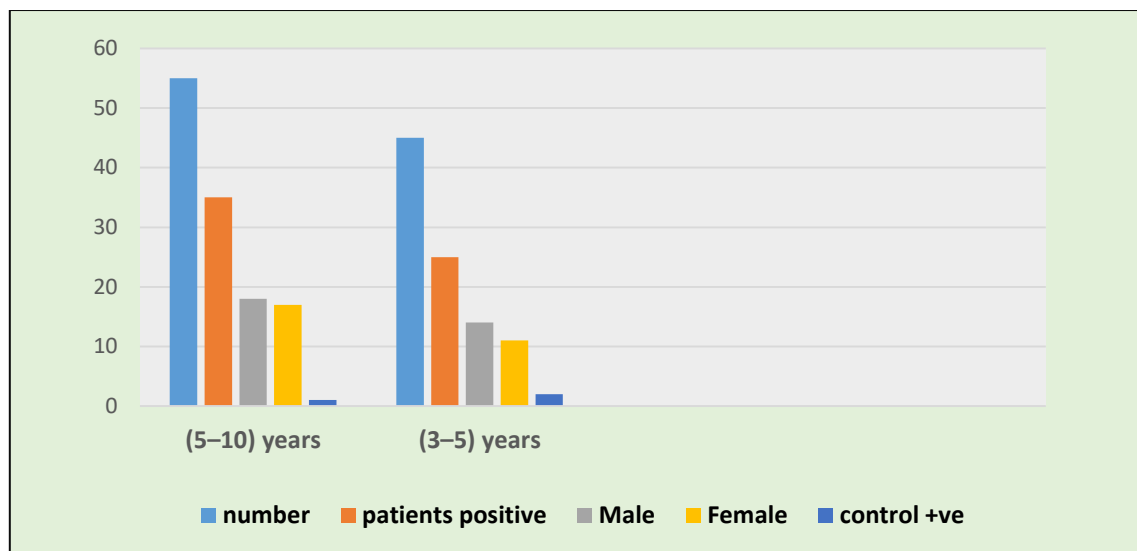


Figure (8): Patient in Baghdad region by RT-PCR results

3.2 Discussion

Usually, patients with Mumps infection first feel loss of taste, with nonspecific symptoms; such as low-grade fever, loss of appetite, and headache. Most of the children included in this current study identified Mumps signs and had swelling salivary glands, or parotid glands under the ear. There were (115) total persons infected with mumps in Baghdad, Mumps occurred in (55) patients and the result of IgG +ve was (31) patients, in the age group (5–10) years, was (13) in males gender while up to (18) in females mostly in the age range (5–10) years. While in outside Baghdad were 46% male, and 30% female a total of 95 patients. A presence of positive IgM results within (19) patients (8 Males and 4 females) within (5–10) years, and (5 Males and 2 females) within (3–12) years, compared with control positive results of (4, and 6) in the same age groups, indicates reinfection, current, or very recent infection.

Due to a positive IgM test result can also observed following Mumps vaccination [13], for this, the IgM assay could help in diagnosis but is not dependent on a confirmatory result. As well as

detecting IgM and IgG helps in detecting acute and previous Mumps infections respectively [12]. This high result can attributed to receiving the vaccine after exposure to the virus will not help to prevent disease, especially when the individual has previously been infected. This result agrees with another study [14]. Also, most of the cases were present in Baghdad, because crowded places increased the infection among the children who hadn't been given the mumps vaccine, or had vaccine after being infected with a virus, and this suggestion is in agreement with [15].

Approximately 27% of Mumps patients had no symptoms or signs of illness, while others had respiratory symptoms, or just non-specific symptoms [16]. Mumps infections are usually considered infectious mostly from a few days before until five days after the parotitis onset. For this, if they had not isolated Mumps patients for five days after their glands began swelling this caused an outbreak of elevated infectious patients, this result agrees and supported by [17].

IgM-positive cases are classified as probable, while RT-PCR by buccal swab specimens

clinically compatible with a positive Real-Time Polymerase Chain Reaction results as confirmed identification. So within this assay, there was efficacy evaluated up to (60) patients from (100), mostly within the male gender (32), while in females (28), and also appeared decline in positive results within the control group. This suggests the PCR assay sensitivity is sufficient for detecting Mumps virus infection in patient specimens without false positive results compared with other laboratory methods. The sample collection was only from a buccal specimen swab, for the detection of viral RNA by RT-PCR method, which was >3 days after the symptom started, and the collection of a swab specimen was perfect for the RT-PCR detection method [18].

Also, this assay was achieved well unrelatedly by the nucleic acid that was extracted by both of ways, manual or automated methods, with diverse groups of PCR reagents. Therefore these facts recommend that the PCR assessment is strong and simply transportable [19]. These results are supported by many factors that increase apportion and rise of infection among children in different regions by malnutrition, late or non-vaccination, and sewage or bad sanitation [20].

4. Conclusion

The early diagnosis and identification of mumps illness are important, for these measures can be taken to minimize and prevent widespread mumps transmission around the community especially among children. Diagnosis by RT-PCR method had significant differentiation between real effective infection and ancient or inactive infection. Most infected patients were either non-vaccinated or had a vaccine after viral infection, so

vaccination is very important for decreasing the infection and the outbreak if given early in a regular system. The most effective factors were irregular vaccination, crowded places, and sewage. Mumps infections are usually considered infectious mostly from a few days before until five days after the parotitis onset. For this, the most important recommendation is to isolate Mumps patients for 5 days after their glands begin to swell, to avoid causing serious complications. There are many factors that increase apportion and rise of infection among children in different regions by malnutrition, late or non-vaccination, and sewage or bad sanitation.

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