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Prevalence of Feline Panleukopenia Virus in Cats in Duhok Province, Iraq

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THE current study targeted evaluation the prevalence of feline panleukopenia virus (FPV) in cats in Duhok province-Iraq, using immunochromatography assay (ICA) as a rapid test, indirect enzyme immunosorbent assay (i-ELISA) and conventional polymerase chain reaction (c-PCR). Moreover, determining the compatibility, sensitivity, specificity and accuracy between the different diagnostic techniques. A total of 100 fecal swab samples were collected from 52 household cats and 48 stray cats in various regions in Duhok province and tested using Rapid test and c-PCR, also 100 blood samples were drawn from cephalic and/or saphenous veins of same cats and tested using i-ELISA. The overall prevalence of FPV was 40%, 66% and 70% using rapid test, i-ELISA and c-PCR technique respectively. The prevalence was significantly higher in stray cats compared with household cats according to all tests used in this study. A moderate compatibility observed between rapid test and c-PCR based on Kappa value (0.440) with sensitivity 57.14%, specificity 100% and accuracy 70% of rapid test compared with c-PCR. Moreover, Fair compatibility between i-ELISA and c-PCR technique based on Kappa value (0.312) with sensitivity 75.71% , specificity 56.66%, and accuracy 70% of i-ELISA compared with c-PCR technique. It has been conclude that FPV is widespread in cats at Duhok province-Iraq. However, stray cats have a significant role in spreading of disease, and the rapid test and i-ELISA need to confirm using c-PCR technique.

Keywords: Feline Panleukopenia virus, ICA rapid test, i-ELISA, c-PCR, Duhok-Iraq.

Introduction

Feline panleukopenia viral (FPLV) disease, otherwise called cat plague and feline distemper, it is a highly contagious often fatal viral disease affecting domestic and wild felids such as cats, mink, raccoons foxes and monkeys [1], tigers and leopards [2,3], jackals and badgers [4] and lions [5]. The disease caused by viral species Carnivore Protoparvovirus 1, from the genus Protoparvovirus within the family Parvoviridae, which divided into two subfamilies, the first called Parvovirinae that can infect wide range of vertebrate animals, whereas the second division called Densovirinae that are isolated from invertebrate animals [6,7]. The family Parvoviridae includes mink enteritis virus (MEV), canine parvovirus type 2 (CPV-2), and raccoon parvovirus (RPV) and other parvovirus of carnivores [6], which depend on the host susceptibility, and on mutations of amino acid sequence in VP2 gene [8]. The FPL virus is mainly transmitted by direct contact with infected cats and their excretions (Nasal discharge, saliva, urine and feces) or indirectly by various fomites contaminated with virus and mechanical vectors such as flies, other insects during worm weather [1, 9, 10]. Furthermore the virus Vertical transmission (trans-placental) from the infected pregnant cats to the fetus via placenta to cause resorption, mummification, stillbirth and abortion

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of embryo [11, 12]. Cats of all ages are affected with FPV, but kittens are more severely infected with the mortality ranges from 90 to 100% in peracute form of the disease [12, 13].

Feline panleukopenia virus has the ability to infect various tissues and body organs, resulting in a range of symptoms. The severity of these symptoms is influenced by different factors related to the host, environmental conditions, and the virus replication ability in highly dividing cells within various tissues [12, 14, 15]. The clinical manifestations of the FPLV in cats include a high fever (40- 41.6°C), anorexia, oculo-nasal discharge, vomiting, abdominal pain, hemorrhagic diarrhea, dehydration, pale of mucus membranes, nervous signs such as (Incoordination, tremors, ataxia, and lateral recumbence). Moreover, disease cats showed eye lesions (Blindness, conjunctivitis, and corneal opacity), as well as mouth lesions [15-17]. Furthermore, infected queens may show infertility or abortion either, dead or mummified fetuses while some kittens may be born with CNS form [14]. In Iraq, FPLV disease was firstly reported in 2016 by Al.Bayati[18].

The clinical manifestations and pathological alterations of FPLV in cats are not definitive to confirm the presence of the disease because they may interfere with other diseases such as feline immunodeficiency virus and feline leukemia virus [19,20], feline calcivirus [21,22] and feline bocavirus or feline astrovirus [23]. Therefore, there are several laboratory techniques developed to confirm FPLV in cats such as viral isolation, hemagglutination assay (HA), immunofluorescence assay (IFA), electron microscopy, and polymerase chain reaction technique [24]**,** virus neutralization and hemagglutination inhibition test [25,26], direct enzyme linked immunosorbent assay as immunochromatography rapid tests [16], and indirect enzyme linked immunosorbent assay and immune chromatography assay [15]. Moreover, conventional polymerase chain reaction (cPCR) technique [27], Real time PCR technique [28], and Multiplex PCR technique [23].

Feline panleukopenia virus was not previously reported in Duhok province, Iraq, and little information's has been provided, Therefore, the present study was preliminary conducted to detect the presence of FPV in cats at Duhok province for the first time, by several laboratory procedures, and also to evaluate the compatibility of these involved methods.

Material and Methods

Ethical approval

This work was ethically permitted by the animal ethics committee of the college of veterinary medicine, university of Duhok, (DR.199611CV) on the 7th of June 2021.

Animals and samples collections

This study included 100 cats from different lifestyle (household cats and stray cats), breeds (Short hair, persian and angora), ages (≥ one years to > one years) and regions in Duhok (Sumil, Zakho and Duhok). During the period from December, 2021 to November, 2022, one hundred fecal swab samples were collected from 52 household cats and 48 stray cats ICA rapid test to evaluate the antigen of the FPV and the all diluent samples of the ICA were tested using c-PCR also to detect the antigen of the FPV. Furthermore, 100 blood samples (2ml of blood) were drawn from all cats via cephalic and/or saphenous veins then keep in plane tubes for separating serum and stored at -20°C until tested using i-ELISA to detect anti-FPV antibodies.

Immunochromatography assay (ICA) Rapid test

This rapid test was performed as an initial approach to detect the specific antigen of the feline panleukopenia virus in fecal swab samples using an immunochromatography assay kit provided by Biotechnology Inc. Elabscience®, USA (Catalog No: E-AD-C063). The assay was done according to manufacturer instructions.

Indirect ELISA (Feline Panleukopenia Virus Ab ELISA)

This test was used to confirm presence of anti-FPV antibodies in the sera. Feline Panleukopenia Virus Ab ELISA kit supplied by DRG International, Inc. DRG®, USA (Catalog No: EIA-2467) was employed. The assay was done as mentioned in manufacturer instructions.

DNA extraction and amplification for conventional -PCR

The 100 fecal swab diluent samples of the ICA were used to extract the DNA using the AcroGene viral Nucleic Acid Extraction kit (AcroGene, USA). The process was performed as mentioned by the manufacturer. Using the Nanophotometer (BioDrop, Germany), regarding to wavelength 260nm the concentration of extracted DNA was ranged between 50.8 - 362.5 ng/ µl. Additionally, the purity of extracted DNA, calculated by ratio of (A260 nm to A280 nm), which was between 1.7 - 1.9.

The amplification of the highly conserved viral protein 2 (VP2) gene of FPV was done by using c-PCR technique. The DNA extracted from clinically and laboratory positive cat was used as a positive control. Furthermore, The extracted DNA from healthy and laboratory negative cat was used as a negative control. The oligonucleotides of specific primers was designed by Aydin and Timurkan [29]. These primers were supplied by (Macrogen Inc. South Korea), which comprising forward primer VP2F (5'-CAGGTGATGAATTTGCTACA-3') and revers primer VP2R (5'-CATTTG GATAAA CTGGTGGT-3'). To identify the positive cats for FPV using the specific primers (VP2F and VP2R), were in approximately band size 640 bp. The c-PCR was performed with a total volume of 20µl, including (2X) master mix 10µl, each primer (VP2F and VP2R) 1µl (10 pmol), template DNA 3µl, and nuclease-free water 5µl. The program setting for the thermocycler (BIO-RAD/ USA) was as follows: predenaturation step 5min at 95°C (1 cycle), denaturation step 30s at 95°C, annealing step 1min at 54°C, and extension step 1 min at 72°C (35 cycles), with a final extension step 1 min at 72°C (1 cycle), according to Aydin and Timurkan [29]. The final PCR products were loaded in a 1.5% agarose gel that was stained with Safe-Red™ dye, and the resulting bands were visualized under UV transillumination (BIO-RAD/ USA).

Comparison between the methods used in this study

The compatibility between ICA rapid test and the c-PCR technique and i-ELISA and the c-PCR technique were assessed based on Kappa value. There was no compatibility between the two tests; if the Kappa value is < 0.00, the compatibility is low; if the Kappa value is ranged 0.0 - 0.20, the compatibility is fair; if the Kappa value is ranged 0.21 0.40, the compatibility is moderate; if the Kappa value is ranged 0.41 - 0.60, the compatibility is substantial; if the Kappa value is ranged 0.61 - 0.80 and the compatibility is almost perfect; if the Kappa value is ranged 0.81 - 1 [30]. Moreover, accuracy, sensitivity and specificity of rapid test and i-ELISA were computed and compared to the PCR technique [31].

Statistical analysis

X^2- test and Kappa value were used by IBM-SPSS Version 22 (Inc., Chicago, USA), to analyze the data in this study. Statistically significant data was determined at the P value ≤ 0.05.

Results

In the current work, the total prevalence of FPV in cats in Duhok province based on ICA rapid test was 40% (40 out of 100) (Fig. 1) , i-ELISA was 66% (66 out of 100) and c-PCR was 70% (70 out of 100) (Fig. 2) (Table 1). The result indicated that the prevalence of FPV in household cat was 32.69%, 51.92% and 55.76%, while in stray cats was 47.91%, 81.25% and 85.41%, using rapid test, i-ELISA and c-PCR technique respectively, these indicate that significantly higher prevalence of FPV in stray cats compared with household cats according to all tests used in this study (Table 2).

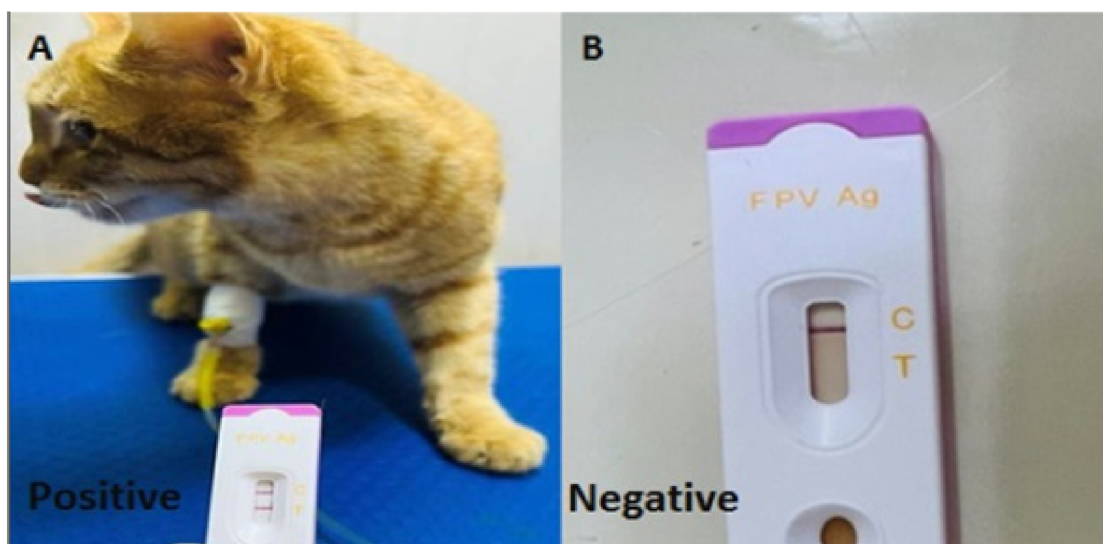

Fig. 1. Fecal swabs examined using immunochromatography assay (Rapid test): A- Cat showed positive for PLV. B- Negative cat for FPV.

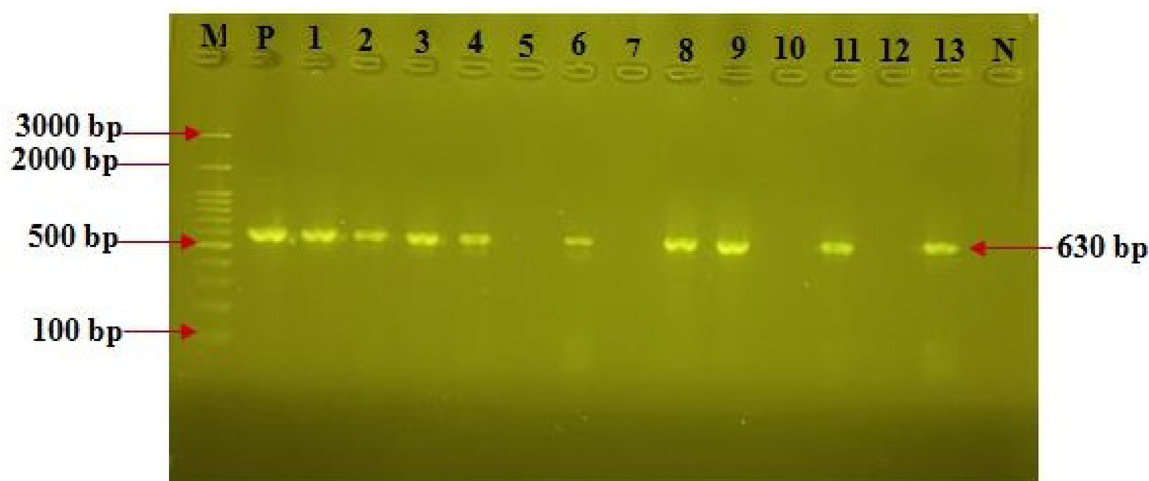

Fig. 2. Gel electrophoresis image showing: lane M) Exact Mark 100-3000bp DNA ladder; Lane P) positive control DNA extracted from cat infected with FPV; Lane 1-11) c-PCR technique detected FPV in approximately band size 630bp except (4, 6, 9 and 11) were negative; Lane N) negative control DNA extracted from FPV-free cat.

TABLE 1. Overall prevalence of feline panleukopenia virus in Duhok province using ICA rapid test, indirect ELISA and conventional PCR technique.

Type of test	No. of tested cats	No. of positive cats	Prevalence
ICA rapid test		40	40%
Indirect ELISA	100	66	66%
c-PCR technique		70	70%

TABLE 2. Prevalence of feline panleukopenia virus according to lifestyle of cats using rapid test, indirect ELISA and c-PCR technique.

Lifestyle	No. of tested cat	Type of test		
		Rapid Test (%)	i-ELISA (%)	c-PCR (%)
Household	52	17 (32.69)^{a}	27(51.92)^{a}	29 (55.76)^{a}
Stray cat	48	23(47.91)^{b}	39(81.25)^{b}	41(85.41)^{b}

A significantly different ($P < 0.05$) were assigned by different superscript letters (a,b).

Moreover, a moderate compatibility observed between ICA rapid test and c-PCR technique based on Kappa value which was 0.440, with sensitivity, specificity and accuracy of rapid test were 57.14%, 100%, 70% respectively compared with c-PCR technique (Table 3). While, fair compatibility showed between i-ELISA and c-PCR technique based on Kappa value which was 0.312 with sensitivity, specificity and accuracy of i-ELISA were 75.71%, 56.66%, 70% respectively compared with c-PCR technique (Table 4).

TABLE 3. Compatibility between ICA rapid test and c-PCR technique based on kappa value, with the calculating the ratio of the ICA rapid test's sensitivity, specificity, and accuracy for FPV diagnosis.

		Conventional PCR technique		
		Infected	**Uninfected**	**Total No.**
ICA rapid test	Infected	40[a]	0[b]	40
	Uninfected	30[c]	30[d]	60
Total		70	30	100

(a)True positive samples , (b)False positive samples, (c)False negative samples, (d) True negative samples. Kappa value was (0.440). Sensitivity= a/(a+c)x 100= 57.14%. Specificity = d/(b+d)x100= 100%. Accuracy =(a+d)/(a+c+b+d) x100= 70%

TABLE 4. Compatibility between i-ELISA and c-PCR technique based on kappa value, with the calculating the ratio of the i-ELISA sensitivity, specificity, and accuracy for FPV diagnosis.

		Conventional PCR technique		
		Infected	**Uninfected**	**Total No.**
i-ELISA	Infected	53 a	13 b	66
	Uninfected	17c	17 d	34
Total		70	30	100

(a)True positive samples, (b) False positive samples, (c) False negative samples, (d) True negative samples. Kappa value was (0.312). Sensitivity= a/(a+c)x 100= 75.71%. Specificity = d/(b+d)x100= 56.66%. Accuracy =(a+d)/(a+c+b+d)x100= 70%.

Discussion

In the current work, the total prevalence of PLV in cats in Duhok province was 40%, 66% and 70% using ICA rapid test, i-ELISA, and PCR technique respectively. This finding is higher when compared with reports mentioned the prevalence of FPV in Iraq. Al. Bayati [18] stated that the prevalence of FPV among cats in Iraq was 38% and 51.1% in fecal samples using ICA as rapid test and PCR technique respectively. The prevalence in Baghdad province was 24% in fecal samples and 21% in blood samples using PCR technique, by using i-ELISA and ICA rapid test the prevalence were 36.1% and 22.2% in serum and fecal samples, respectively[15,32]. Furthermore, various studies worldwide indicated varying prevalence rate of FPV in cats using diverse laboratory tools such as in Saudi Arabia was 4.48% using indirect fluorescent antibody test (IFA) [33], in different province of Turkey was 10% and 25%, using c-PCR and RT-PCR respectively [27,29], in Iran was 34% using ICA [34], United Arab Emirates (UAE) was 2.2% using ICA [35], in Indonesia and Bangladesh was 72.7% and 18.375 respectively [36,37], in Egypt was 35% and 43 using ICA and c-PCR respectively [38], in Korea, Germany and Italy was 36.36%, 48.7% and 73.5% respectively using real time PCR [24,28,39], and in China was 37.06% using multiplex PCR [23]. The variations in prevalence of FPV in variety of regions and countries were caused by varying management strategies, environmental circumstances, effective diagnostic procedures utilized in various studies, and the presence and/or absence of other parameters including the age, physical and immunological status of the host [27,38-40].

This study observed that the prevalence of FPV was significantly higher in stray cats than in household cats. This result agrees with the finding of Bukar-Kolo et al. [41]; Radhy and Zenad [32]; Amoroso et al. [39] and Abdel-Baky et al. [38]. This probably might owing to frequent expose of stray cats to the virus in the environment because the virus more resist to adverse environmental condition. Thereby, the stray cats with clinically and subclinically infection play an important role in spreading the virus to healthy household cats

as they shed the virus for long periods and/or when it was peregrinate in the houses searching on food [18,42]. The most of household cat owners manage their cats' health by vaccinating and preventing contact with stray cats [43].On the other hand the seroprevalence of FPV was significantly higher in household cats in compared with stray cats. The elevation of antibodies against FPV in household cats maybe due to belonged to an implied regular vaccination program, and the stray cats did not exhibit a significant rise in the antibodies against FPL virus because of their mode of living that may have contributed to these cats' exposure to FPL virus antigen, which may be limit increase in the specific antibody formation [15]. Furthermore, Jenkins et al.[44] stated that no significant different among stray and pet cats, also between indoors and outdoors cats.

This study indicates that moderate compatibility observed between ICA rapid test and c-PCR technique based on Kappa value. In spite of, the ICA is rapid, low cost and an easy field diagnostic test used in the fields, since it is usually relevant for veterinarians and farmers as well. It needs other confirmatory diagnostic test due to suspected negative results that might be associated with this test [45]. The sensitivity, specificity and accuracy of ICA rapid test were 57.14%, 100%, 70%, respectively compared to c-PCR technique. These results considered consistent with Mosallanejad et al. [34]; Islam et al. [46] and Al.Bayati [18]. In contrary, Esfandiari and Klingeborn [47] they mentioned that the evaluation of ICA revealed a high sensitivity and specificity that may reach to 95.8% and 99.7%, respectively.

Moreover, results observed that fair compatibility between i-ELISA and c-PCR technique based on Kappa value. It is probable due to the different of the target for the two methods (In i-ELISA the target is the antibodies, while in c-PCR technique the target is the antigen), also most of the infected cats have antibodies against FPV, because some cats may be immunized with hyper immune serum or vaccination, other may have acquired maternal immunity and/or outcome from natural exposure to FPV infection [12, 28]. In this study sensitivity, specificity and accuracy of i-ELISA were 75.71%, 56.66%, 70% respectively compared with c-PCR technique. These finding disagreements with Awad et al. [16] they mentioned that the sensitivity, specificity and accuracy of ELISA were 88%, 100%, and 94.5% respectively. Raheena et al. [48] stated that polymerase chain reaction (PCR) assay is considered as a highly sensitive, specific and rapid technique for confirmative diagnosis of feline panleukopenia virus (FPV) infection in cats.

Conclusion

This study states that FPLV disease was circulated at Duhok province, Iraq with a high prevalence rate within household and stray cats with significantly higher in stray cats. The results of ICA rapid test and i-ELISA need to confirm using c-PCR technique. Furthermore, strengthening cats management practices through responsible cats ownership and applying a carefully planned program for FPV vaccination of both stray and household cats as a disease control measure was advised.

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Conflict of Interest

No conflicts of interest exist, according to the authors, with the publishing of this work.

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انتشار فيروس طاعون القطط في القطط في محافظة دهوك، العراق

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استهدفت الدراسة الحالية تقييم نسبة انتشار فيروس طاعون القطط في القطط في محافظة دهوك- العراق، باستخدام الاختبار الكروماتوغرافي المناعي كاختبار سريع واختبار الممتز المناعي غير المباشر وتقنية تفاعل البلمرة المتسلسل التقليدي. كما اشتملت هذه الدراسة على تحديد التوافق والحساسية والنوعية والدقة بين التقنيات التشخيصية المختلفة. تم جمع 100 مسحة براز من 52 قطة منزلية و 48 قطط سائبة في مناطق مختلفة في محافظة دهوك وفحصها باستخدام الاختبار السريع وتفاعل البلمرة المتسلسل التقليدي، كما تم سحب 100 عينة دم من الوريد الرأسي والوريد الصافن من نفس القطط المفحوصة، وتم فحصها باستخدام اختبار الممتز المناعي غير المباشر. أظهرت النتائج ان معدل الانتشار الكلي لفيروس طاعون القطط كانت 40 ٪ و 66 ٪ و 70 ٪ باستخدام الاختبار السريع واختبار الممتز المناعي غير المباشر و تقنية تفاعل البلمرة المتسلسل التقليدي على التوالي. كانت نسبة الانتشار أعلى معنويا في القطط السائبة مقارنة بالقطط المنزلية وفقًا للاختبارات المستخدمة في هذه الدراسة. لوحظ توافق معتدل بين الاختبار السريع و تقنية تفاعل البلمرة المتسلسل التقليدي اعتمادا على قيمة كابا (0.440) مع الحساسية التي بلغت 57.14 ٪ والنوعية التي بلغت 100 ٪ والدقة التي بلغت 70٪ للاختبار السريع مقارنة بتقنية تفاعل البلمرة المتسلسل التقليدي. فضلا عن ، لوحظ التوافق المعتدل بين اختبار الممتز المناعي غير المباشر و تقنية تفاعل البلمرة المتسلسل التقليدي اعتمادا على قيمة كابا (0.440) مع الحساسية التي بلغت 75.71 ٪ والنوعية التي بلغت 56.66 ٪ والدقة التي بلغت 70 ٪ لاختبار الممتز المناعي غير المباشر بتقنية تفاعل البلمرة المتسلسل التقليدي. استنتج من هذه الدراسة، الى الانتشار الواسع لفيروس طاعون القطط في محافظة دهوك - العراق. أذ أن للقطط السائبة دور كبير في انتشار المرض، و ان قلة التوافق بين الاختبارات تشير إلى أن نتائج الاختبار السريع واختبار الممتز المناعي غير المباشر تحتاج إلى تأكيد باستخدام تقنية تفاعل البلمرة المتسلسل التقليدي.

الكلمات الدالة: فيروس طاعون القطط ، الاختبار السريع ICA ، اختبار ELISA غير المباشر ، تقنية PCR التقليدية ، محافظة دهوك - العراق.