

STUDY OF CONTAGIOUS ECTHYMA IN LAMBS OF BASRAH PROVINCE , IRAQ

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ABSTRACT

Contagious ecthyma have been detected and diagnosed in local lamb breeds 3-6 months old of both sexes . The study was conducted to examine 941 local lamb breeds in Basrah, province , Iraq represents eleven (11) flock groups. One hundred (100) local lamb breeds shows different clinical manifestations belong to contagious ecthyma. Twenty five (25) clinically healthy local lamb breeds considered as controls. Diseased lambs show different clinical manifestations such as Anorexia, depression and dullness , Unable to sucking or graze , However, Orf lesions was seen in the form of papules, pustules, vesicles, and scabs which indicated in all diseased animals, Moreover, Orf lesions distributed around mouth commisure and muzzle , Furthermore, lesions was detected at upper and lower part of the lips , additional, Orf lesions was seen on upper and / or lower eyelids . Fissuring lesions were also detected . Moreover, a slight , lesions were also detected on coronets, ears, anus, and vulva in (6%) of diseased animals. Data concerning clinical examinations of diseased lambs show a significant increase in body temperature, respiratory and heart rate of diseased lambs than in controls. The results of hematological examinations of diseased lambs infected with Orf and controls indicated leucocytosis due to lymphocytosis, Moreover, the rate of erythrocyte sedimentation of red blood cells indicated a significant increase in diseased lambs than in controls. On the other hands, The results of the acute phase response also indicated a significant increase in both Haptoglobin values and Fibrinogen time in diseased lambs compared with controls. The results of PCR on gel electrophoresis show that the ORFV virus has 147 base-pair specific PCR amplicons were detected, In addition, Sequence analysis of submitted *Orf virus*

amplified *GFR gene*. The raw nucleotide sequences of all samples were processed by FinchT.V 1-4 version software for trimming the unwanted sequences and bases with low sequence quality (lower than 20% signal intensity). Furthermore, The evolutionary history was inferred using the Neighbor-Joining method, Since, The optimal tree with the sum of branch length = 0.02625234 is shown. (above the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 79 positions in the final data set. Evolutionary analyses were conducted in MEGA7 . Results of the histopathological examinations show papillomatus hyperplasia of epidermis as well as a numerous immature hair follicles in the proliferation epidermis and proliferation of sebaceous glands, However, an area of apoptosis of structural molecules were also detected , Furthermore, Masses of areas of proliferative sweat glands with large areas of laminated vacuolated structure and deposit tissue like structures in the dermis was determined, However a dark stained blackish deposit hair follicles with some dilated hair follicles and inflammatory cells were also observed, Moreover, scab like formation above the epidermal cells in the upper epidermal layer was seen microscopically. It have been concluded that contagious ecthyma seems to be an endemic viral disease at Basrah providence , Iraq, reflect high morbidity rate which might resulted in a recoded economic losses. However, Secondary bacterial infection or myiasis of affected parts were always follow resulting in more disease complications, Therefore programmed annual vaccination is advised.

INTRODUCTION

Contagious ecthyma, or orf, is a highly contagious ubiquitous disease of sheep, goats, characterized by maculopapular and proliferative lesions affecting the skin around the mouth, nostrils, interdigital regions, teats, and the oral mucosa, The disease caused by the epitheliotropic Orf virus a member of the genus Parapoxvirus. (1,2). Contagious ecthyma also has been reported in other wild and domestic ruminant and in humans (3,4). It's an important disease and common throughout the world wherever small ruminants are raised as farm animals(2).

Orf, is an old English for rough, usually affects the mucocutaneous junctions of the muzzle and lips, although lesions within the mouth affecting the gums, palate, and tongue can occur, especially in lambs and kids. Less frequently, lesions occur on the eyelids, feet, and teats. Lesions of orf progress from papules to pustules and then to thick crusts (5). The infection in sheep and goats is generally known as orf, contagious ecthyma, infectious labial dermatitis, scabby mouth, contagious pustular dermatitis, and sore mouth (6).

Orf virus is a member of parapox viruses belongs to the family poxviridae. This genus also includes pseudocow pox virus and bovine papular stomatitis virus (6,7). The family Poxviridae is characterized by viruses with linear double stranded deoxyribonucleic acid (DNA) molecule of 130 to 300 kilobase pair (kbp) with a hairpin loop at each end (8). Viruses belonging to this family replicate entirely in the cytoplasm because their virions contain enzymes that synthesize messenger ribonucleic acid (mRNA).

In sheep and goats, the disease mostly occurs in young animals 3-6 months old, although neonatal lambs and kids aged 10-12 days old can be severely affected as well (9). However, older sheep may also carry the virus without showing lesions and introduce the disease into susceptible flocks (10,2).

Grazing of coarse pastures or stubbles may predispose to infection with scabby mouth as oral abrasions increase the potential for the virus to gain entry (11). Since, The orf virus infects damaged or scarified skin through rough grazing and replicates in regenerating epidermal keratinocytes.

According to World Organization of Animal Health, Orf is a modifiable and zoonotic disease transmitted from animals to humans(12).

Contagious ecthyma was detected and diagnosed in Basrah province , Iraq, therefore, The main aims of the current study are, clinical and hematological studies and explore the main clinical manifestations of the disease showed by diseased sheep with molecular identification of the causative virus via PCR technique, Histopathological study of the orf lesions and Evaluation of acute phase response of diseased animals via evaluation of haptoglobin and fibrinogen .

MATERIALS AND METHODS

Animals and Study design :- The study was conducted to examine 941 local lamb breeds 3-6 months age and from both sexes in Basrah province , Iraq, represents eleven (11) flock groups. One hundred (100) local lambs breeds shows different clinical manifestations belong to contagious ecthyma. Twenty five (25) clinically healthy local lambs bleeds considered as controls. Complete clinical examinations was applied to all animals, However , coprological and blood smear examinations have been done to exclude blood parasitic infection and gastrointestinal parasite infestations use a routine laboratory methods .

Collection of samples and hematology:- Ten milliliters of blood (10 mL) were drained from each animal by jugular vein puncture and from these (2.5) milliliter of blood mixed with EDTA used to determine Total erythrocyte count (TRBc), Hemoglobin concentration (Hb), packed cell volume (PCV), and Total leukocytes count (TLC), (Hematology analyzer, Genex, USA), Moreover differential leukocytes count were done using Giemsa stain blood smears method according to Weiss and Wardrop (13) ,Furthermore erythrocytes sedimentation rate (ESR)were also estimated according to Stevens *et al* (14) .

DNA extraction and Polymerase chain reaction (PCR):-

Viral DNA was extracted from lip scrape tissue samples of clinically infected with lambs with Orf. The samples were collected in sterile containers under aseptic conditions and transported as soon as possible to post graduate laboratory unite at college of Veterinary Medicine ,University of Basrah, Iraq, and stored at -20°C refrigerator until use for viral genomic DNA extraction.

Viral genomic DNA extraction:- Viral genomic DNA was extracted from lip scrape tissue by using (Genomic DNA extraction tissue kit. Geneaid. USA). The PCR technique was used to detect the polymorphisms of the orf gene using forward and reverse primers TCAACTGCGGCTTCTTCAAC) (GCGTTTCGTTTTCTGTACTCC) respectively) , The primers were synthesized by(BIONEER, Korea). Amplification reaction was performed using a DNA thermo-cycler, 1.5% agarose gel electrophoresis was stained with ethidium bromide under UV light(15, 16).

Sequence :- The positive PCR products (12 samples) of ORFV were sequenced (BIONEER, Korea).

Estimation of Haptoglobin (Haptoglobin ELISA method)and fibrinogen :-

According to manufacture instructions,(Biotechnology co -china) Serum was used for evaluation of ELISA Haptoglobin. the stop solution changes the color was measured at 450 nm using a spectrophotometer. Moreover, Fibrinogen, was estimated according to manufacture instructions of (Biolabo / France) using, Blood mixed with trisodium citrate using plasma).

Histopathological examinations :-

Histological preparation of the external organs (lips) was done according to Bancroft and Steven,(19). Since, After fixation of specimens by 10% NBF for 5 days, these specimens were washed by tap water for 2-4 hours to remove excess of fixatives and the specimens were transferred into the classical steps of dehydration, clearing, Infiltration and Embedding, cutting and finally staining with Hematoxylin and Eosin.

Statistical analysis:-Data was analyzed and the significant difference between diseased and control group were statistically analyzed using student *t*-test (SPSS) program (18).

RESULTS

Diseased lambs show different clinical manifestations such as Anorexia, depression and dullness (81%), Unable to sucking or graze(77%), However, Orf lesions was seen in the form of papules, pustules, vesicles, and scabs which indicated in all diseased animals, Fig. 1. Orf lesions distributed around mouth commasure and muzzle was found in (76%), Furthermore, Orf lesions was distributed at upper and lower part of the lips (69%), additional, Orf lesions was seen on upper and / or lower eye lids (12%), Fissuring lesions (10%), Fig.2, Moreover, a slight , Orf lesions was also detected on coronets, ears, anus, and vulva in (6%) of diseased animals .Table 1.

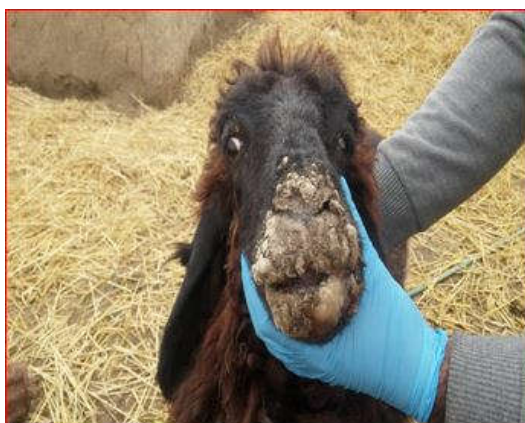


Fig. 1: Photograph of a sheep showing different proliferative orf lesions with thick scab.



Fig.2: Fissuring of lips.

Table(1): Clinical manifestations of infected lambs with Orf

<i>Clinical signs</i>	<i>Diseased sheep n=100</i>	<i>%</i>
Anorexia,depression and dulness	81	81%
Unable to sucking or graze	77	77 %
Orf lesions in the form of Papules, pustules, vesicles, and scabs	100	100%
Orf lesions distributed around mouth commensure and muzzle	76	76%
Orf lesions distributed at upper and lower part of the lips	69	69%
Orf lesion on upper and / or lower eye lids	12	12%
Fissuring lesions	10	10%
Orf lesions on coronets, ears, anus, and vulva	6	6%

Data concerning clinical examinations of diseased sheep show a significant ($p<0.05$) increase in body temperature, respiratory and heart rate of diseased animals than in controls Table 2.

Table(2): Body temperature, respiratory and heart rate of diseased lambs infected with Orf and controls

Parameters	Controls n=25	Diseased sheep n=100
Body temperature C°	39.4± 0.08	41.3 ± 1.2**
Respiratory rate/ min	25± 0.75	55.6± 8.2**
Heart rate/ min	75.2±0.74	91.8± 10.21**

*Values are mean ± standard error of mean. ** (P<0.05).*

The results of hematological examinations of diseased lambs infected with Orf and controls indicated leucocytosis due to a significant ($p<0.05$) increase in the number of total leukocyte count and a significant ($p<0.05$) increase in the absolute number of lymphocytes, (lymphocytosis), Moreover the rate of erythrocyte sedimentation of red blood cells indicated a significant increase ($p<0.05$) in diseased sheep than in controls. Table 3.

Table (3): Hematological parameters of diseased lambs infected with Orf and controls

Parameters	Controls n=25	Diseased lambs n=100
RBC $\times 10^6$	7.93±1.46	7.97±0.35
Hb g/dl	13.23 ± 1.77	13.6±0.3
PCV %	32.61 ± 4.64	33.93±1.09
Thrombocytes $\times 10^3$	9704.57±568.34	9604.42±448.23
TLC $\times 10^3$	11.43±1.54	14.84±0.75**
Neutrophils $\times 10^3$	4.39± 0.16	4.45±0.84
Lymphocytes $\times 10^3$	5.55± 0.42	8.91±0.35**
Monocytes $\times 10^3$	0.53± 0.07	0.54±0.06
Eosinophiles $\times 10^3$	0.56 ± 0.13	0.61±0.14
Basophiles	0.08±0.04	0.08±0.02
ESR mm/24hr	6.36± 4.72	18.32± 5.42 **

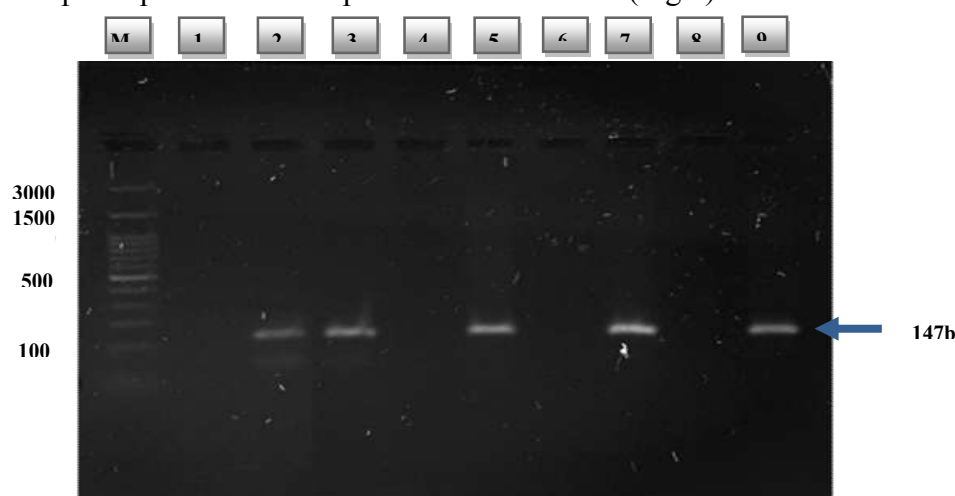
*Values are mean ± standard error of mean. ** (P<0.05).*

The results of the acute phase response also indicated a significant increase ($p<0.05$) in both Haptoglobin values and Fibrinogen time in diseased lambs compare with controls .Table 4.

Table (4): Haptoglobin values and Fibrinogen time of diseased lambs infected with Orf and controls

Parameters	Controls n = 25	Diseased lambs n = 100
Haptoglobin g/dl	0.024 ± 0.011	0.035± 0.007**
Fibrinogen time / Sec	27.18 ± 8.31	35.65± 7.53**

The results of PCR on gel electrophoresis show that the ORFV virus has 147 base-pair specific PCR amplicons were detected (Fig.3).



PCR product of Orf virus GIF gene (1.5%) agarose gel, M: ladder, lane 2, 3, 5, 7 and 9 positive results ; 1, 4, 6: negative results; 8, negative control

Sequence analysis of submitted *Orf virus* amplified *GFR gene* was conducted by BLAST alignment tool (Blastn) accessed through <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, the homologues species/isolates were chosen by highest identity percentage with best query cover and lowest E-value. The raw nucleotide sequences of all samples were processed by FinchT.V 1-4 version software for trimming the unwanted sequences and bases with low sequence quality (lower than 20% signal intensity). The identity percentage, accession number and nucleotides variation presented in the Table 5.

Table (5):Sequence analysis :

			country		Location	Substance	Type	
Orf virus 1	Orf virus strain AH-GY13 GM-CSF/IL-2	100	China	MF770655	-- -- -- -- --	----	-----	316 to 407
Orf virus 2	Orf virus strain AH1704 GM-CSF/IL-2	100	China	MF489147	-- -- -- -- --	----	-----	316 to 406
Orf virus 3	Orf virus strain B029,	99	Germany	KF837136	1 1 7 6 3	A-- -T	Transversion	117135 to 117226
Orf virus 4	Orf virus strain F94.848R GM-CSF/IL-2	99	Finland	JF773684	3 4 2	A-- -T	Transversion	315 to 406
Orf virus 5	Orf virus isolate OrfV/KPM/TN/Sheep/12 GM-CSF/IL-2	100	India: Tamil Nadu"	KY077479	-- -- -- -- --	----	-----	327 to 408
Orf virus 6	Orf virus isolate OrfV/MEC/TN/Goat/13 GM-CSF/IL-2	100	India	KY077478	-- -- -- -- --	----	-----	327 to 408
Orf virus 7	Orf virus strain AH1701 GM-CSF/IL-2	99	China	MF489146	9 9	N-- --	insertion	315 to 407
Orf virus 8	Orf virus strain F94.848R GM-CSF/IL-2	100	India	KY077479	-- -- -- -- --	----	-----	328 to 408
Orf virus 9	Orf virus strain AH1402 GM-CSF/IL-2 i	99	China	MF489139	3 6 9	C-- A	Transversion	316 to 407
Orf virus 10	Orf virus strain AH1505 GM-CSF/IL-2	99	China	MF489141	3 6 1	A ...C	Transversion	315 to 406
Orf virus 11	Orf virus isolate ORFV/Hyderabad/25/Sheep/2006 GM-CSF	100	India	MF414634	-- -- -- -- --	----	-----	327 to 406
Orf virus 12	Orf virus strain AH1604 GM-CSF/IL-2	100	China	MF489143	-- -- -- -- --	----	-----	312 to 406

Multiable alignment:

Table 6:Multiable alignment of orf virus GFR gene

M7: Alignment Explorer (abid.masi)

Data Edit Search Alignment Web Sequencer Display Help

DNA Sequences Translated Protein Sequences

Species/Group Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	12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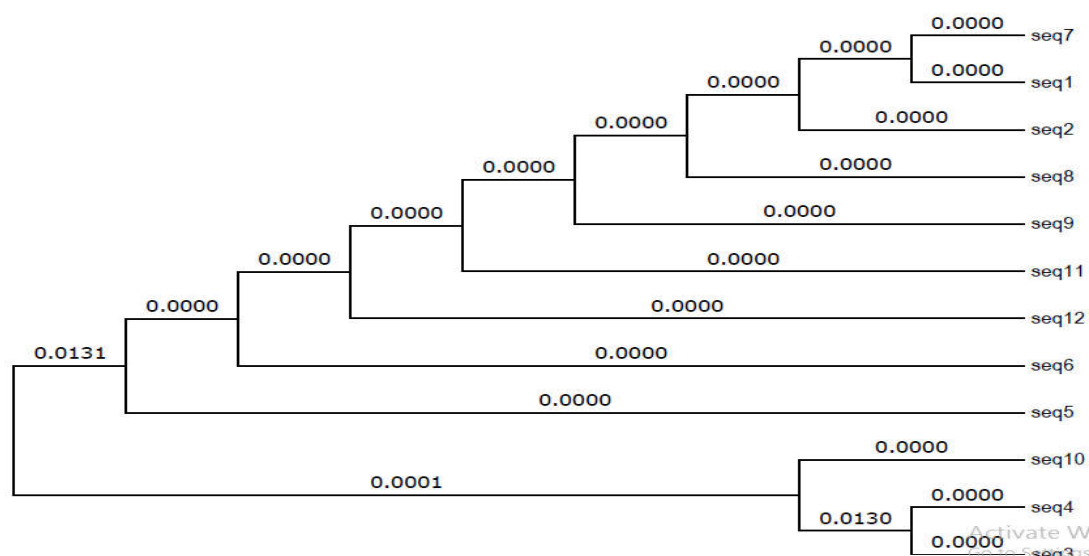


Fig. 4: Phylogenetic tree of different Orf virus based on the nucleotide sequences of GFR genes by using the neighbour-joining methods in Mega7 software.

Submission on National Center for Biotechnology Information (NCBI):

The Orf virus isolates submitted in gene bank under the accession numbers:

seq1 MK950771
seq2 MK950772
seq3 MK950773
seq4 MK959356
seq5 MK959357
seq6 MK959358
seq7 MK959359
seq8 MK959360
seq9 MK959361
seq10 MK959362
seq11 MK959363
seq12 MK959364

Results of the histopathological microscopical examinations of lips of diseased lambs show papillomatus hyperplasia of epidermis (Fig 5), as well as a numerous immature hair follicles in the proliferation epidermis (Fig 6) and proliferation of sebaceous glands (Fig 7). However, an area of apoptosis of structural molecules were also detected , Furthermore, Masses of areas of proliferative sweat glands with large areas of laminated vacuolated structure and deposit tissue like structures in the dermis was detected, However a dark stained blackish deposit hair follicles with some dilated hair follicles and inflammatory cells were also observed, Moreover, scab like formation above

the epidermal cells in the upper epidermal layer was seen microscopically (Fig.8- 10).

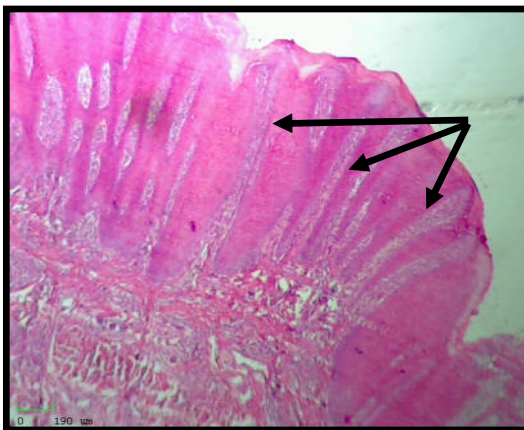


Fig. 5: Panillomata's hyperplasia of epidermis 4X



Fig. 6: Numerous immature hair follicles. 10 X

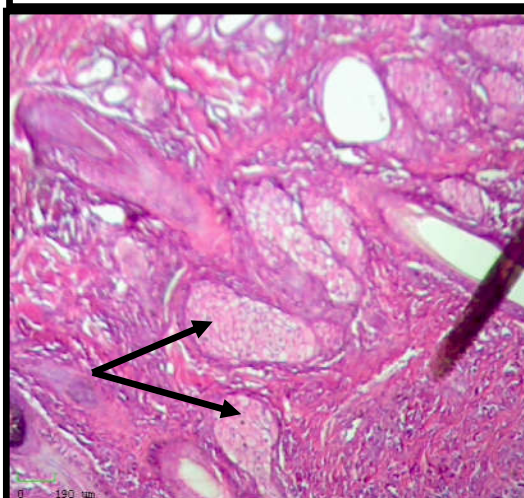


Fig. 7: proliferation of sebaceous glands . 10X



Fig.8: Areas of apoptosis formed of structural molecules(non cellular). 10X



Fig. 9: Masses of areas of proliferating sweat glands .

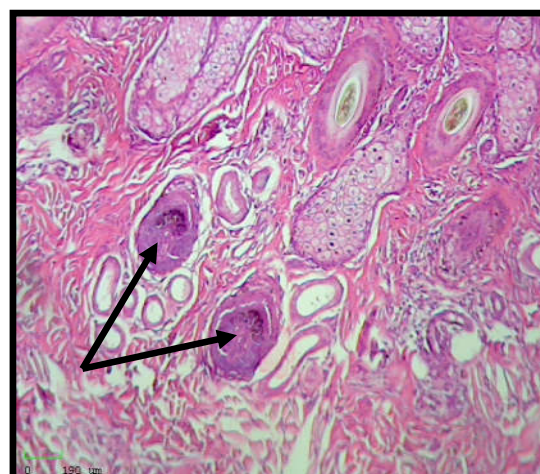


Fig.10: Hair follicles with dark stained blackish deposit. 10X

Contagious ecthyma is a highly contagious, viral skin disease that affects sheep, goats and some other domesticated and wild ruminants, although a number of proprietary treatments as well as homeopathic preparations are available, (22). Since infection occurs by direct contact with the virus which is highly resistant and can survive in the environment for more than one year, In addition the disease occurs most commonly in young ages, However, occasionally seen and registered also in older sheep whom mostly grazing of rough pastures those had a cut stalk of cereal plants that may predispose to infection with scabby mouth as oral abrasions increase the potential for the virus to gain entry (2). In this study, we presented the first clinical report of Orf virus in small ruminants based on molecular identification and histopathological diagnosis at Basrah province , Iraq .

The disease has a high morbidity rate whilst, the mortality rate is low, as a result, less attention has been given by the owners. However, the loss of condition of the diseased animals due to the disease significantly might expose to danger the market value (11). Over the study period, we have recognized the lack of adequate diagnosis and effective treatment of clinical cases which is the main contributing factor for the occurrence of disease outbreaks at any time points.

Diseased lambs show different clinical manifestations which are mentioned by (2,11,12,23), As, Orf infection can be clinically manifested as simple lesions around the commisure of the mouth, These lesions usually begin as erythema, followed by papules, pustules, which develop into brownish dry scabs. The time span of these stages is usually four to six weeks, more or

less. Nevertheless, under certain circumstances as in young malnourished lambs the disease may take a chronic form, which might take more than 4-6 months to heal completely, in such chronic cases, the lesions might spread to other parts of the skin regions (24).

It had been shown previously, that contagious ecthyma is endemic all over the world, but its less reported in the literature because of its low morbidity and minimal economic consequences (25). In Basrah province, more cases of contagious ecthyma in sheep and goat populations have been observed over the past several years, but no specific vaccination or control programs has been applied to control and eradicated this disease.

Contagious ecthyma may become a serious problem in young, stressed, immuno-suppressed or overcrowded animals (26). The differential diagnosis of the diseases causing crusts in small ruminants like contagious ecthyma, pox and PPR which have, in some cases, similar clinical symptoms may be a problem, with the development of molecular biology, the PCR technique has met the demands for specific and sensitive diagnosis of orf virus infection in the field specimens from affected animals (25,27). The present study has demonstrated for the first time the characteristics and specifications of lambs contagious ecthyma in Basrah province , Iraq.

In general contagious ecthyma will commonly affects animal lips, However mouth and surrounding skin were also targeted and harmed, Furthermore the disease can also affect the face, feet and even the udder skin of lactating ewes, As the causative virus will cause superficial sores, which then will crusted and finally scabbing over and then falling down. Moreover, the underlying skin heals without scarring, where, this cycle takes approximately 4-5weeks or may be less, Thereby, lambs will lose its condition as they are reluctant to eat and it is too painful for ewes to feed suckling lambs (2) . On the other hand, Hosamani, *et al* (6), Emphasized that, Orf lesions promotes through the stages of erythema papule, vesicle, pustule, scab formation, and finally resolution, Since, Orf pustules will develop within a few days, However, when it will ruptured ulcers and a thick scab will formed which shed within 3-4 weeks, leaving no scar tissue, Although the immunity is solid but could only last for eight months, In addition, there was an antibody

response to the virus, Furthermore, these sores may become infected by opportunistic bacteria, causing further infection (11).

In this study, it was indicated that the infection of Orf virus is common around mouth and lips of the diseased animals. This is due to the close confinement and grazing habit of the animals which causes the formation of minor abrasions on the mouth and lips of the animal during feeding. A very low incidence of the virus was also observed at other parts like on the teat and udder of the animal particularly in nursing animals in which the infected lambs could possibly be the source of infection during suckling (2,6).

The diagnosis of contagious ecthyma is based upon the finding of large proliferative lesions in the animal body, In the current study the clinical diagnosis of ORF in sheep show wart-like lesions which were distributed in the skin of the lips, gums and muzzle . This agreed with the study which stated that the clinical signs of ORF include multifocal to coalescing papillary, proliferative and ulcerated lesions in the epidermis of the muzzle and lips moreover, In some cases the lesions appear on and in the nostrils, around the eyes, on the thigh, coronet, vulva, udder and axilla (28,29).

The traditional methods of diagnosis which depends on the characteristic clinical signs could be inaccurate, but virus isolation and culturing is thought to be a gold standard method of confirmation, although it is time-consuming (8). With the development of molecular biology, PCR technique has become widely used to amplify the desired genomic fragments of tissue specimens, and it has become a powerful tool in molecular diagnosis. This method is recommended for proper identification of the pathogen through gene amplification using specific forward and reverse primers (10). Workers in various different parts of the world have reported contagious

ecthyma as a common outbreak of sheep and goats and indicated that PCR is a quick confirmatory test for Orf virus (21).

Results of the current study indicated leukocytosis due to significant increase in lymphocytes number (Lymphocytosis) this agreed with (13) , whom mentioned that leukocytosis can be indicated as a reaction to different

infectious, inflammatory, However in those conditions, This reaction could be mediated by several molecules, which are released in response to stimulatory events that include growth or survival factors such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, c-kit ligand, or adhesion molecules and various cytokines, Moreover, because the demands on the leukocyte producing tissues in the bone marrow have increased to the point at which there is an insufficient number of mature cells for delivery into the circulation, as the infection subsides, the number of younger forms and the total white cell count decrease and ultimately return to normal , during the period of repair following an inflammatory reaction, the monocytes may increase in number, and subsequently the lymphocytes will become more numerous (30), on the other hand , Certain types of infection specially viral types are characterized from the beginning by an increase in the number of small lymphocytes unaccompanied by increases in monocytes or granulocytes, and such lymphocytosis is usually of viral origin, However , moderate degrees of lymphocytosis are encountered in certain early acute and chronic infections , (31), Furthermore , (32) added that some infectious diseases, caused by some viral infection were associated with the appearance of unusually large lymphocytes (atypical lymphocytes).

In the current study the diagnosis with histopathological features reveal, papillomatus hyperplasia of epidermis, as well as a numerous immature hair follicles in the proliferation epidermis and proliferation of sebaceous glands, However, an area of apoptosis of structural molecules were also detected , Furthermore, Masses of areas of proliferative sweat glands with large areas of laminated vacuolated structure and deposit tissue like structures in the dermis was detected, Same results are also indicated by (33,).Whom describe the histopathological lesions, As, they mention that, the crusts around nose and lips. These lesions are the late stage of the disease, are formed after rupture of vesicles and pustules, and are responsible for the common name scabby mouth, Moreover, Blood may be incorporated into crusts following severe exudation and inflammation that can damage vessel walls secondarily, However, The blood may contribute to the darkly colored crusts , In addition, The epidermal hyperplasia (acanthosis), ballooning degeneration, vesicle and neutrophils accumulating in the vesicle, which subsequently

results in the formation of a pustule. Free red blood cells are present in the epidermis to the left of the vesicle. Epidermal hyperplasia, upward movement of the pustule, and rupture of the vesicle or pustule contribute to crust formation. Furthermore, (34) added that, hyperplasia of the epidermis and follicular infundibula results in a papillary appearance of the surface that is further accentuated by stacks of exudative crust overlying the congested and inflamed dermal papillae. However, Segment of ballooning degeneration of keratinocytes in the stratum granulosum near the edge of a lesion were also detected, As well, Affected keratinocytes are swollen with pale eosinophilic cytoplasm, The keratohyalin granules are peripheralized, but the nucleus remains in the center of the keratinocyte. One or more large, acidophilic cytoplasmic viral inclusion bodies typical of parapoxvirus infection.

Others (35) were also added that, The histopathological examinations of the proliferative verrucous lesions of the affected animals revealed severe epidermal hyperkeratosis and hyperplasia. There were degenerative changes within the stratum spinosum, with numerous swollen, Vacuolated cells having pyknotic nuclei . Intraepidermal aggregates of inflammatory cells were present, and the formation of intracytoplasmic eosinophilic inclusion bodies was present in vacuolated necrotic prickle.

It had been shown that, lesions induced by viral challenge of mildly abraded skin, indicated that the virus does not establish in the damaged epidermis, but replicates in the cells of an underlying replacement epidermal layer derived from the walls of the wool follicles. The skin reaction consists of a cellular response with necrosis and sloughing of the affected epidermis and underlying stratum papill are of the dermis. Healing is then completed with the formation of a third epidermis derived from the deeper portions of the wool follicles. The previous cutaneous infection did not prevent re-introduction of the disease, even in the same area of the skin, although the lesions were less severe and persisted for a shorter period (2,11).

Results of this study also show a significant increase of acute phase response, Represented by a significant increase of both haptoglobin and fibrinogen . The acute phase response refers to the non-specific and complex innate reaction that occurs shortly after tissue injury, Since , Proinflammatory cytokines are released initially at the site of an insult and are responsible for the induction of local and systemic defenses (36). Of the inflammatory cytokines, the interleukin-6, Tumor necrosis

factor-alpha (TNF- α), and interleukin-1-beta are the major mediators of acute phase protein (APP) synthesis in the liver, which is the main site of APP synthesis, although non hepatic sites have also been recognized(37). Serum concentrations of positive APPs increase by over 25-30% during an acute phase response. However, Some APPs decrease in concentration such as albumin and transferrin, and are referred to as negative APPs. Acute phase proteins function to further activate the immune system, enhance phagocytosis, and clear the products of inflammation. Moreover, APPs may be more sensitive than leukocyte counts as markers of inflammation, are more stable than cellular components, and the assays can be performed on previously frozen and stored serum or plasma. As , it were thought that APPs also have a faster response than changes in WBC counts in situations where new WBCs must be generated by the bone marrow. (38).

Sequence analyses used based on the GFR gene used to determine the ORFV virus. Genomic sequences of ORFV were assembled into a contiguous sequence . The identities of the ORFV strain with other ORFV strains were 99%–100% at the nucleotide level, presences transversion A-C nucleotide (NCBI). (5).

Although the disease is endemic in most parts of the world, there are few descriptions of Orf virus strains and comparisons of these strains between them. Details of 31 Orf virus strains, whose sequence of the envelope gene (B2L) has been reported before.(39).

It had been shown that, Full-length B2L gene encoding for immunogenic major envelope protein from most ORFV isolates was amplified by PCR and the amplicons (1206 bp) were cloned and sequenced. Since , Comparative sequence analysis revealed an open reading frame of 1137 nucleotides (nt) encoding a polypeptide of 378 amino acids (aa). Indian isolates were highly related amongst themselves with sequence identity of over 97% at the nt and aa level. Further, they showed 97-98% sequence identity with sequences of other ORFV isolates from around the world, whereas, 94-95 and 82.7-83.8% sequence identity was observed, respectively, with pseudocowpox and bovine papular stomatitis viruses, the other members of the genus, Phylogenetic analysis also showed that these Parapoxviruses from sheep and goats are closely related to other orf viruses reported worldwide (6). Moreover, The ORFV strain from the Thi-Qar Province showed a close relationship with other strain in Asia . Among strains originating in sheep and goat was closer to strain in India, Germany and Finland Analysis also showed that ten ORFVs were more closely related to the

other region from Iraq (NCBI). The phylogenetic tree based on the GFR gene showed the nine sheep ORFVs and three sheep ORFVs formed distinctly separate branches. Analysis of the phylogenetic trees based on nucleotide sequences of each gene of ORFV. Multiple alignment of the nucleic acid sequence showed that gene presences transversion A-C.(40).The availability of genomic sequences of three sheep ORFVs aids in understanding of the diversity of orf virus isolates in this region and can assist in distinguishing between orf strains that originate in sheep .

دراسة مرض الحميقاء الساري في حملان محافظة البصرة، العراق

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الخلاصة

شخص مرض الحميقاء الساري في الحملان المحلية ومن كلا الجنسين بعمر ٣-٦ اشهر .أذ شملت الدراسة فحص ٩٤١ من الضأن المحلية مثلت احد عشر من قطاعان ضأن محافظة البصرة ، العراق . عشوائياً تم اختيار ١٠٠ من الحملان المحلية والتي اظهرت علامات سريرية متعلقة بالمرض كما اختير ٢٥ من الحملان المحلية السوية سريرياً عدو كمجموعة سيطرة . اظهرت الحملان المصابة علامات سريرية مثل انعدام الشهية والاكثاب والبلادة،عدم القدرة على الرضاعة أو الرعي،فضلا عن ذلك فقد لوحظت الافات المرضية بشكل حطاطات، بثرات ، حويصلات وندب في جميع الحملان المصابة .لوحظت الافات المرضية حول الفم وفي الشفاه العليا والسفلى، وفي الجفون العليا والسفلى، كما لوحظت افات التشقق ايضا في الحملان المصابة وتواجدت افات مرضية بشكل قليل جدا حول الاكليل والأذنين والشرح والفرج (٦%).كما عانت الحيوانات المريضة من ارتفاع معنوي في معدلات درجات حرارة الجسم وضربات القلب وتردد التنفس. أوضحت نتائج الفحوصات الدموية ارتفاع معنوي في العدد الكلي لخلايا الدم البيض بسبب الارتفاع المعنوي للعدد المطلق للخلايا اللمفية فضلا عن حدوث ارتفاع المعنوي في سرعة تثفل كريات الدم الحمر في الحملان المصابة بالمرض بالمقارنة مع مجموعة السيطرة ومن ناحية أخرى فقد بينت نتائج استجابة الطور الحاد وجود ارتفاع معنوي في معدلات الهابتوكلوبين ووقت منشيء الليفين في الحملان المريضة بالمقارنة مع حملان مجموعة السيطرة . بينت نتائج فحص تفاعل البلمرة المتسلسل في الهلام الكهربائي ان الفيروس المتسبب عن الحميقاء الساري له ١٤٧ من القواعد الزوجية . تم اختيار الأنواع المتماثلة / العزلات حسب أعلى نسبة هوية مع تغطية أفضل للاستعلام وأدنى قيمة E.كما تمت معالجة متواليات النيوكليوتيدات الخام لجميع العينات بواسطة برنامج إصدار FinchT.V 1-4 لتقليص المتواليات والقواعد غير المرغوب فيها بجودة تسلسل منخفضة (أقل من كثافة الإشارة بنسبة ٢٠%). فضلا عن

استنتاج التاريخ التطوري باستخدام طريقة الجار - الربط ، حيث يتم عرض الشجرة المثلى مع مجموع طول الفرع = ٠.٠٢٦٢٥٢٣٤. (فوق الفروع). حساب المسافات التطورية باستخدام طريقة أقصى احتمال مركب وهي بوحدات عدد البدائل الأساسية لكل موقع أذ تضمن التحليل ١٢ سلسلة من النيوكليوتيدات وتضمنت اتجاهات الكودون الاول+ الثاني + الثالث + غير المرمز . تمت ازالة جميع المواقع الغامضة لكل زوج متسلسل حيث أن هناك مامجموعه ٧٩ وظيفة من مجموع البيانات النهائية. واجريت التحليلات التطورية في MEGA7.

بينت نتائج الفحوصات النسجية المرضية تضخم الورم الحليمي للبشرة وكذلك العديد من بصيلات الشعر غير الناضجة في انتشار البشرة وانتشار الغدد الدهنية ، ومع ذلك ، تم اكتشاف منطقة من موت الخلايا المبرمج للجزيئات الهيكلية. فضلا عن تحديد كتل مناطق الغدد العرقية التكاثري مع مساحات كبيرة من هياكل مغلفة وأنسجة رواسب مثل الهياكل في الأدمة، ورواسب داكنة اللون مع بعض بصيلات الشعر المتوسعة والخلايا الالتهابية. وشوهدت مماثلات الندب فوق خلايا البشرة في الطبقة العليا من الجلد . استنتج من هذه الدراسة أن مرض الحميقاء الساري قد يكون متوطناً في محافظة البصرة وقد يعكس اصابات مرضية مرتفعة قد تؤدي الى هلاكات فضلا عن اصابة الحيوانات المريضة بالنغف أو الإصابات الجرثومية الثانوية والذي قد يعقد عملية الشفاء عليه ينصح بتباعد برامج سنوية ممنهجة للسيطرة على المرض.

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