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Contagious Ecthyma in Sheep

A Graduation Project Submitted to the College of Veterinary Medicine, Al-Qadisiyah University in Partial Fulfillment of the Requirements for the Degree of Bachelor Of science in Veterinary Medicine and Surgery

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Summary.

Orf disease is a highly contagious , zoonotic viral skin disease affects sheep , goats and some other domesticated and wild ruminants. The causative agent is *parapoxvirus* belongs to *poxviridae* family , *chordopoxvirinae* sub family .

Orf disease occurs most commonly in lambs 3-6 months of age when at pasture, while lambs 10-12 days of age and adult sheep can be harshly exaggerated , and epidemics relating the lips and face of new lambs and the udders of the ewes.

Morbidity proportion of the disease can be actual great , imminent 100% , nonetheless the mortality degree in simple cases hardly surpasses 1% . In this regard, minor staphylococcal contagion is a common incidence and mortality rates may be since 20% to 50% in dirtied herds.

Contagious ecthyma disease showed highly spread of lesions as vesicles , pustules , ulcers and papillomatous proliferative lesions at the skin of lips , nostrils , eyes , tail , abdominal wall , udder and thigh regions .The clinical signs in lambs less than one month of age were more sever than older lambs and adult which were accompanied with granulomatous lesions of dental pad and deformity with separation of teeth that causing starvation and sever dehydration due to inability of sucking and eating . Infected lambs showed fever (41-42C°) , anorexia , depression , there has been high heart rate ranged (90 – 120 min) respiratory rate ranged (40 – 80 min) associated with abnormal respiratory sound (exaggerated breath sound) and dyspenea .

Histopathological pictures showed characteristic pathological changes as acanthosis , hyperplasia of stratum basale , hyperkeratosis of keratinized layer , thickening in the wall of hair follicles in the dermis layer . There has been esinophilic cytoplasmic inclusion bodies in swollen cells of the stratum granulosum layer with increased infiltration of inflammatory cells , there has been vasodilatation of blood vessels supply to affected area.

The disease is easily diagnosed from lesions and symptoms confused with other skin diseases , but it requires laboratory confirmation, which includes serological and molecular techniques, or electron microscope examination.

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Abbrev.	Terms
Bpsv	Bovine popular stomatitis virus
CD	Dendritic cells
CE	Contagious Ecthyma
CPE	Cytopathic effect
CTL	Cytotoxic T cells
ELISA	Enzyme – linked immunosorbent assay
Pcpv	Pseudocowpox virus
PCR	Conventional polymerase chain reaction
PPVs	parapox virus
Pvnz	Parapoxvirus of red deer in New Zealand
qPCR	Qualitative polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RT- PCR	Real Time PCR
Sppv	Squirrel parapoxvirus

1.1. Introduction.

Orf disease is a extremely contagious, zoonotic, viral skin disease affects sheep, goats , other domesticated and wild animals. It is also known as Contagious pustular dermatitis (CPD), contagious ecthyma , infectious labial dermatitis and scabby mouth, or sore mouth .(1). Orf has been found worldwide, it has been reported in many countries including North and South America, New Zealand, Finland, Germany, Norway, Japan, Italy, United Kingdom, Western Australia, middle east in Iraq , Iran , Saudi Arabia and other Arabians countries , South Asian countries and recently Antarctica (2).

The causative agent of the disease is a *parapox* virus (Family *Poxviridae*, Sub-family *Chordopoxvirinae*) (3) . Genus *parapoxvirus* has five species and three tentative species according to International Committee on Taxonomy of Viruses (ICTV) , these are *Orf virus* (Orfv) , *Bovine popular stomatitis virus* (Bpsv), *Pseudocowpox virus* (Pcpv) , *Parapoxvirus of red deer in New Zealand* (Pvnz) and *Squirrel parapoxvirus* (Sppv) . (4).

Orf disease occurs most commonly in lambs 3-6 months of age when at pasture, while lambs 10-12 days of age and adult sheep can be harshly exaggerated , and epidemics relating the lips and face of new lambs and the udders of the ewes. Outbreaks arise at every time but they are greatest shared in dry conditions when ewes are at pasture, or in penned sheep being fed from feed troughs. (5). Morbidity proportion of the disease can be actual great , imminent 100% , nonetheless the mortality degree in simple cases hardly surpasses 1% . In this regard, minor staphylococcal contagion is a common incidence and mortality rates may be since 20% to 50% in dirtied herds . The mortality happens, particularly in early drinking lambs, because of a frequency of dehydration and starvation, as the pain and alteration of the lips and mouth preclude the lamb from sucking. (1).

The infection is spread by direct and indirect contact from infected animals or by contact with infected tissue or saliva containing the virus (6).

Orf virus is actual resilient to opposing environments and physical issues except ultraviolet light. It may continue in shed scabs from one year to ten years but quickly loses contamination when exposed to rain on grass (7).

Sheep can also transmit the virus without viewing lesions and present the disease into vulnerable flocks (8).

Cuts are most usually limited in and about the mouth and nostrils. The lesions in clinical duration , improvement finished the erythematous , macula , papule , vesicle , pustule and scab foundation . In straightforward contagious ecthyma (CE) , natural salvage takings three to six weeks, with cracking of scab materials dirty with virus . Prolongation of the infection and an increase in severity are nearly associated with secondary bacterial infections.(9).

The histopathological changes of the effected skin with Orf disease which include acanthosis , hyperplasia of stratum basale layer , hyperkeratosis , thickening in the granulosum layer , vaculated of spinocyste cytoplasm , increased hyperplastic cells of stratum basale and esinophilic cytoplasmic inclusion bodies in swollen cells of the stratum granulosum layer (10, 11).

Orf is indistinguishable clinically from other diseases like sheep pox, foot-and-mouth disease (FMD), bluetongue, peste des petits ruminants, Staphylococcus dermatitis, papular stomatitis and ulcerative dermatosis. Hence, from the zoonotic point of view, there is an increased hazard to the owner and veterinarian alike unless the true nature of such skin disease is clearly differentiated from other dermatopathies . This demands in developing a rapid, sensitive diagnostic tool for differential diagnosis. (12).

Although the disease is easily diagnosed from lesions and symptoms confused with other skin diseases , but it requires laboratory confirmation, which includes serological and molecular techniques, or electron microscope examination .

1.2. Amis of the study .

- **Evaluation of the some clinical signs associated with Orf virus infection.**

- **Study of important histopathological changes of Orf virus infection in sheep.**

2. Contagious Ecthyma

Contagious ecthyma (CE) also known as Orf, infectious labial dermatitis, scabby mouth, or sore mouth (13). It is a common epitheliotrophic highly contagious, zoonotic viral skin disease of sheep , goats , camels , dogs , cats and squirrels. The disease also takes a zoonotic latent , while it is more of an occupational hazard to people occupied with animals (e.g., farmers , animal careers , veterinarians).(14).

The causative agent of the CE is Orf virus is the prototype member of the *Parapoxvirus* genus within the *Poxviridae* family(15).

Clinically the illness firstly dowries itself as papules (raise of the skin) that growths to blisters (fluid-filled pouches) or pustules before encrusting. These cuts are start in the skin of the lips. They can feast everywhere the outside and inside of the mouth , face , lips , ears , vulva , scrotum , teats and feet , frequently in the interdigital region. The infection is extent by straight and unintended contact from infected animals or by interaction with infected tissue or saliva covering the virus (6).

Infected animals commonly show a decrease in feed consumption and certain become unhappy , anorectic and febrile. Difficulties of contagious ecthyma comprise secondary bacterial infections (respiratory, gastrointestinal, integumentary), myiasis, mastitis and lameness. In sheep and goat, the disease generally transpires in early animals 3-6 months old, while neonatal lambs and kids aged 10-12 days hoary can be harshly affected (16).

The disease occurs normally in a mild form in sheep and goats with high morbidity and significant productivity losses . Mortality ranges from 10% to 90% in lambs and kids (17) and mortality may be increased due to secondary bacterial infections (18).

2.1. History.

Backgrounds of the term (Orf) are indistinct , but some foundations originate it from Old Norse *hrūfa* ("crust on a wound, scab") (19 , 20).

Additional cradle originates it from the Ancient English *orfcwealm* ("murrain, any infectious disease of livestock") , from Orf ("cattle") + *cwealm* ("destruction") . Inconsistently, although "orf" may suggestion its origin to a word denotation "cattle, orf does not obviously infect cattle (21).

In Iraq , the virus has been isolated for the first time , characterization and diagnosis by electronic microscopy by (22) during sever outbreaks in sheep in the province of Nineveh , in the village of Hulileh .

In Saudi Arabia ,the disease was known since the beginning of the eighties in the sheep and goats , the disease was considered endemic in the local and foreign sheep and goats in the kingdom (23).

2.2. Replication

The replication of *parapox virus,* the duplication of the additional poxviruses, happens in the cytoplasm of infested cells , This is permitted by the occurrence of a complete transcriptional system inside the *poxvirus* core , which can pledge mRNA synthesis proximately after infection and without host protein synthesis. There are three dissimilar phases of gene expression: early , intermediate and late. The successive expression of these gene classes is beneath the regulation of phase-specific promoters and transcription factors, and the expression of each reliant on prior expression class is of proteins of the preceding class (24).

Educations with Orf virus and *pseudocowpox virus* have revealed that *parapox virus* DNA replication initiates (4-8 h) post infection and remains to (25-

36 h)., and that the first virus-encouraged polypeptides can be noticed preliminary at (10 h.) Both Orf virus and *pseudocowpox virus* particles look at (12-18 h.) and they are shaped until at minimum (48 h.) (25).

2.3. Physico – chemical properties

Orf virus is actual hardy to environmental situations. In gasping scabs the virus is tremendously steady, and pasture, stables or kraals, may endure infected for months or uneven years(26).

The Orf virus isolate was held at 37°C, a 1.5 Log units decrease in the titer was evidenced by the first day, then, gradual decreasing till inactivation was complete by the tenth day. When the viral isolate was held at 56°C, the decrease in viral activity was much accelerated , with a bulk of the virus 2.7 Log units being inactivated within the first five minutes and the complete inactivation was achieved within 25 minutes. heating for 30 min. at 60°C completely inactivated (27).

Scabs dried over sulphuric acid, powdered, and stored in well stoppered sealed glass tubes in an ice box retained potency for at least 32 months . Infectivity is resistant to organic solvents and desiccation (28).

(29) recorded that the attendance of the virus crusted gasping retain it active for long episodes of up to 23 years at a temperature 7 C º. Likewise , the virus does not misplace its effectiveness or influenced when showing to freezing or melting of four consecutive sessions, but on the contrary, its titer increase due to deliverance of the virus from infected laceration cells.

(30) also studied the survival of the virus without freeze for a period of more than three months and noted that the decline in the titer of the virus was $10^{1.2}TCID_{50}$ to $10^{1.5}TCID_{50}$ for different isolate strains.

The virus dose not lose its effectiveness by strong ultrasonic vibration (1.5 Am for 10 min) , but the virus titer increased due to liberation from the ruptured cells because

these vibrations and inhibits the virus occurred when exposed to ultrasonic rays and gamma rays . (13).

The virus is very sensitive to chloroform and loses its effectiveness, and little affected when treated by ether (31).

Treatment of the virus by pure ether in concentration of 20% for 18 hours in $4C^{o}$, the degree titer of the virus is reduced $10^{1}TCID_{50}$ and when treated with pure chloroform by 40 % for a period of one hour in $4C^{o}$, the titer of the virus is reduced $10^{3}TCID_{50}$ .The virus is sensitive to acidic PH 3 , loses its effectiveness in tissue culture cells when exposed for a period of one hour , also the virus affected by the alkaline PH 9-10 and loses a little of its ability to replicate in tissue culture cells when exposed for one hours(22).

The effect of disinfectants on the virus , it is possible to destroy the effectiveness of the virus when use of disinfectants , include 2 % formalin and Iodophors (29).

3. Epidemiology

3.1. Occurrence

The Orf virus is endemic , zoonotic and has a worldwide spreading and sources an infectious skin disease recognized as contagious ecthyma in humans ,small ruminants and other wild ruminants (15).

Orf happens commonly and is one of the greatest significant viral diseases causing excessive economic damage in small routine (32, 33).

Genetic heterogeneity of the Orf virus separates socialize in diverse geographic regions counting Europe, the Middle East, the United States, Africa, Asia, Alaska, South America, Canada, New Zealand and Australia has been verified later the dawn 19th century and has been stated from greatest sheep or goat-raising areas (34).

The infection is extent by direct and indirect interaction from sick animals or by interaction with infected tissue or saliva comprising the virus (6). Banquet of infection can arise by direct communication or finished contact to contaminated feeding troughs and similar fomites containing wheat stubble and thorny plants. The viruses are sometimes communicable to humans after nearby contact with skin lesions of infected animals or handling virus-contaminated materials, so the infections are consequently classed as zoonoses (35).

Orf disease transpires supreme commonly in lambs 3-6 months of age when at pasture, while lambs 10-12 days of age and adult animals can be harshly affected, and outbreaks linking the lips and face of young lambs and the udders of the ewes also are common. Outbreaks occur at some time but they are most shared in dry conditions when the sheep are at pasture, or in penned sheep being nourished from feed troughs. (5).

Young animals are more susceptible to Orf infection, so that the warm months after the lambing season attract attention due to a high infection rate. Mortality in young animals is also higher due to starvation, immunosuppression and secondary infection up to 93% (36, 37).

In humans , veterinarians, wool shearers, abattoir workers, and also nonprofessional persons such as farmers' children and housewives, Muslims in the feast of sacrifice, visitors to zoological gardens and persons who slaughtered their animals for traditional activities are at particular risk (38).

3.2. Morbidity and Mortality

The morbidity of the disease may reach 100% and mortality rate related to Orf is usually low, but it may be very high in small ruminants, especially when bacterial or fungal secondary infections occur (15). In some animals, infection may remain subclinical; however, occasionally and especially in young animals, case fatality may reach up to 80% (14).

Difficulties of contagious ecthyma embrace secondary bacterial infections (respiratory, gastrointestinal, integumentary), myiasis, mastitis and lameness (16).

Secondary staphylococcal infection associated with Orf disease is a recurrent incidence and mortality rates may its array be from 20% to 50% in polluted herds. The mortality occurs , particularly in young sucking lambs, due to a prevalence of dehydration and starvation , as the pain and distortion of the lips and mouth preclude the lamb from sucking. The lambs exaggerated by the mouth form or with strawberry foot rot show considerably compact evolution routine (1 , 9).

Earlier the eradication of screwworm flies (*Cochliomyia hominivorax*) from the USA, Orf was measured a foremost problem for sheep and goat producers because the screwworm larvae attacked Orf lesions , ensuing in secondary bacterial infections

and high mortality. Later the eradication of the screwworm fly from the USA, mortality due to Orf has been abridged significantly. Conversely, Orf remains to be a nuisance,

and outbreaks of the disease with extraordinary morbidity continue to occur on a yearly basis (39).

3.3. Transmission

Scabs that decrease off from curative lesions comprise virus and persist highly infectivefor long periods in dry conditions , but existence of the disease in a flock may be the outcome of chronic lesions that happen for long stages on individual animals. Infection can be from environmental perseverance of the virus or from infected sheep. Extent in a flock is very quick and befalls by contact with other affected animals or by contact with contaminated inanimate objects, such as feed troughs, ear-tagging pliers (40).

An epidemic of lesions on the tails is noted in suggestion with the practice of docking instruments (41).

It has stayed presumed that natural infections on pasture are the outcome of invasion of the virus after skin injury induced by prickly plants or stubble; submission of a viral suspension to scarified skin is the recognized method of persuading orf . But , an outbreak has happened in large group of lambs placid from several farms and elated in a vehicle over a historical of 23 hours . There was no indication of injury to their mouths. (5).

3.4. Risk factors

Orf virus is actual resilient to opposing environments and physical factors excluding ultraviolet light. It may persevere in shed scabs and in lambing huts from one year to ten

years but quickly misplaces infectivity when visible to rain on pasture (7 , 42).

Orf virus is resistant and continues on farm material and the ground for months to years (43). Sheep may also transmit the virus without viewing grazes and present the disease into vulnerable flocks (8).

Infection suppers quickly in a flock , with maximum animals flattering affected within a few weeks. Outbreaks will previous for 6–8 weeks , and normally do not reemerge until there is a new crop of disposed lambs. Survival of infection between outbreaks is presumed to be in the form of virus limited in scabs. Though, although infectivity is reserved in scabs that are dry for protracted periods, infectivity in rainy material is speedily lost (44).

Highly lethal strain of orf virus may have been accountable for the cruelty of the lesions, the clinical and epidemiological characteristics of the orf eruptions in west Texas perhaps suggest that separate intrinsic factors within the Boer breed frolicked a influencing role. In sheep and goats, separate or breed genetic vulnerability and immune faults have been assumed to be contributing factors in orf virus persistence and advance. (45, 46).

Maintenance of the infection and an rise in harshness are nearly associated with secondary bacterial infections (1 , 47,48). Certain infected animals become transporters and shed the virus for a long period. The virus continues in the environment and residues infective for years to susceptible animals and humans, too (49, 50).

Orf virus has been widely examined over recent years, owing to its zoonotic rank and aptitude to cross-infect other species by intermittently evasive mechanisms, that the virus has advanced to familiarize and grow in the attendance of an vigorous immune response and this benefits to explicate the ability of the virus to repeatedly reinfection the same host. Disclosure of animals to pressure or immunosupression as outcome of treatment or main viral infection can accentuate the severity of disease (10 , 18).

3.5. Economic importance

The disease harvests a slight delay excluding when it distresses young sucking lambs with associated lesions on the teats and udders of their ewes. Harm from lamb mortality and secondary mastitis in these circumstances can be important (5).

The disease himself is not deadly, it is stressed that secondary infections may occur because the disease baskets the nutrition of the animals and cause loss of weight. It is recommended that development of the necrosis bacteria in ulcerative lesions can cause sepsis (51, 52).

The economic impact of the disease on sheep farmers due to decreases in production and also has a considerable negative effect on animal welfare (37).

3.6. Zoonotic importance

Orf is zoonotic is identified to source nodular and papillomatous lesions in farmers, veterinarians and butchers who have had interaction with infected livestock and their foodstuffs. The period of the lesions series from 4 to 9 weeks , but standard restorative may be prolonged due to problems (28).

The ways of infection for humans embrace accidental abrasions, cutting, shearing, slaughtering and milking (53). The disease in humans tends to be benign and self-limiting. It commonly manifests as a small ulcer or nodule on the hand or finger (54).

Skin lesions due to Orf are dramatic but benign and resolve spontaneously, except in immunocompromized conditions (55).When Orf is not kept in mind, the disease can be misdiagnosed as more serious conditions such as cutaneous anthrax, leading to overtreatment and also unnecessary medical procedures (43).

The incubation period is generally 3-7 days. The lesions slowly progress from papule, vesicle, shallow annular ulcer, scab, and to healed skin with little or no scarring. Six stages of orf, each of which approximately lasts for one week, are

named as maculopapular, target, acute, regenerative, papillomatous and regressive (56).

The lesions are most often localized on hands, arms or face. Solitary lesions are more frequent than multiple lesions. Duration is from 4 to 9 weeks (57). Healing is complete without scars, but secondary infections may retard healing. Fever, swelling of the draining lymph nodes or blindness following an eye infection is seen rarely. *Erythema multiforme*, toxic erythema or allergic reaction, as well as blisters on arms, body, face or mouth in association with contagious ecthyma have been reported (28).

4. Pathogenesis

Parapoxvirus **imitate in epidermal keratinocytes and confined lesions headway through platforms of papule, vesicle, pustule and scab to determination. The disease is self-limiting in unfussy cases , and the scabs rind off the skin frequently sendoff no scar. Principal lesions can be austere and proliferative, and occasionally continued explosion of the epithelium leads to dense wart-like outgrowth. Secondary complications cause lesions to become ulcerative and necrotic without scab formation, which intervals healing (15, 58). Scabs progress within one week and tenacity in 4-6 weeks, but there have been cases of obstinate long fixed Orf infections in goat kids that lasted three months (59) and six months (60).**

Reproduction of virus is regulated to multiplying epithelial cells and consequently damage to the skin or mucous membranes typically leads infection . Within 24 hours of trauma to the skin , re-evolution of the epidermis arises from the limits of the area of damage and from epidermal cells surrounding persisting hair or wool follicles . By 48 hours after infection vacuoles mature in the cells of the restoring epidermis, and after about 5 days reformist intracellular oedema and cytoplasmic swelling consequences in ballooning degeneration, attended by infiltration with polymorphonuclear neutrophils, lymphocytes and plasma cells (61) . Intracytoplasmic inclusions can be realized in infected cells. Wholly, these changes are related with penetrating local erythema and the formation of small vesicles that then develop into pustules. The stratum corneum covering the pustule ruptures, with ultimate formation of a scab that is firmly devoted and interditgiated with proliferative epidermis. Exclusion of the scab at this stage fallouts in bleeding from the raw, spongy base. While papillomatous growths are not a feature of experimentally persuaded orf , they frequently progress in ordinary orf and may become widespread. They entail of noticeable pseudo-epitheliomatous hyperplasia and granuloma development , which may persevere for several weeks or uniform in some cases many months. Determination of straightforward lesions results in complete re-epithelialization, which leaves no scar, a course that takings about 6 weeks. Lesions

exclusive the mouth do not formula scabs but look as elevated reddened or grayish areas enclosed by an intensely hyperemic zone.(14).

4.1. Immunity

Studies of the skin immunohistological of both principal infected and re-infected sheep have revealed that there is an increase of neutrophils , T cells, B cells and dendritic cells original and together to orf virus-infected epidermal cells (62, 63).

Lesions of Orf virus re-infection are lesser and tenacity faster than primary lesions. This recommends that the host immune response is actual in controlling the magnitude of virus replication. The immune and inflammatory reaction of sheep to cutaneous infection with Orf virus has been studied in skin biopsies , in blood , and in lymph difficult the site of infection (64).

Proliferation of epidermal , seen as dejected progresses or plugs penetrating the dermis (rate formation), is a histological article of Orf , mostly primary lesions. The host reply is characterized by an initial invasion of neutrophils followed by an accretion of dendritic cells, CD4+ T cells, CD8+ T cells and B cells together to and fundamental Orf virus infested epidermal cells. The rate and strength of these cellular changes in the dermis parallels the occurrence of virus in the epidermis and the clinical evolution of the disease. CD4+ T cells are more plentiful than CD8+ T cells at the lesion position , while a quantity of both subsets are stimulated throughout infection. An uncommon feature of the Orf lesion is the thick network of MHC-class II+ dendritic cells together to infected cells. Dendritic cells are best characterized as antigen giving cells that initiate or maintain immune replies. In human skin, the histology of the Orf lesion is totally similar to that in sheep. CD8+ cytotoxic T cells (CTL) are assumed to be significant in host anti-viral immunity by murder virus infected cells via a MHC class-I pathway. During Orf virus reinfection, CD8+ cytotoxic T cells (CTL) were confirmed in the skin and afferent lymph, and revealed to be started at the site of infection (65).

Chapter four……..……..……..Pathogenesis

Analysis of cytokine in the lymph plasma difficult the infection site and concealed from cells in the lymph (in culture) specified a rapid manufacture of the inflammatory cytokine IL- 1β and the chemokine IL-8 after re-infection, and a late production of GM-CSF, IL-2 and IFN-γ (66).

Infected sheep harvest antibodies precise for four or five immunodominant antigens. Murine monoclonal antibodies distinguishing the 39kDa and 42kDa covering proteins, the 10kDa supposed fusion protein and uncharacterized 22kDa

and 65kDa antigens have been labeled, and these can be used to differentiate between the dissimilar *parapoxviruses* (67).

Gathering and weakening in the number of T and B cells parallels the occurrence of virus (measured as virus antigen) in infected epidermal cells. CD4+T cells are the major T cells in the skin in both crucial and re-infection lesions. In principal infections, CD8+ T cells and B cells are slow to gather compared to CD4+ T cells, and γδ T cells are more abundant than in re-infection lesions. In primary lesions there is indication of considerable virus replication, and epidermal thickening and down growth into the dermis (rete formation) is mutual . Virus duplication is less marked in re-infection lesions, which tenacity more rapidly than primary ones and there is less indication of rete creation. The manufacture of these GM-CSF, IL-2 and IFN-γ by lymph cells linked with the two mountains of lymphoblasts chronicled after re-infection. Remarkably, in lymph plasma demanding the re-infection site, GM-CSF and IL-8 declined after an first rise, and only the second (later) peak of IL-2 and IFN-g was sensed associated with the lymph cell biphasic reply (54).

5. Clinical signs

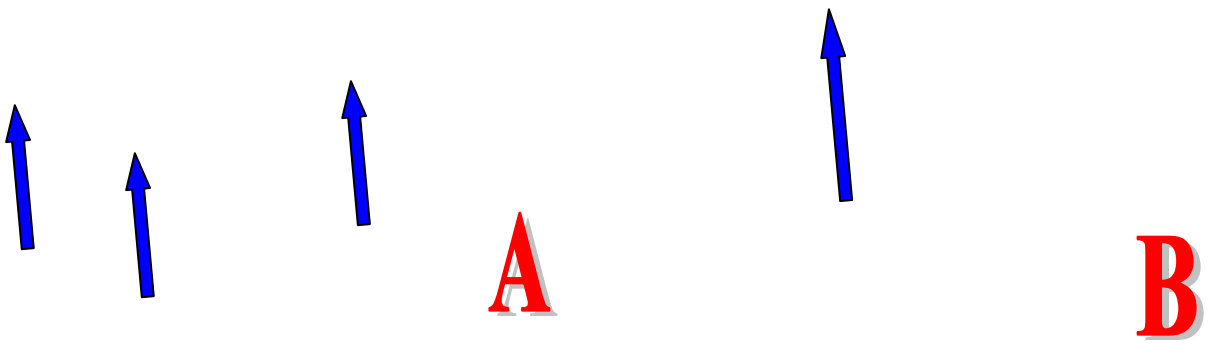
Orf virus is epitheliotropic, which incomes that it has an empathy for the skin; infection happens by direct contact. The period of gestation is relatively short. Vulnerable animals usually mature the principal signs of the disease 4 to 7 days after experience that continues for 1 to 2 weeks or for longer periods ((68).

Skin lesions due to Orf are dramatic but benign and resolve spontaneously, except in immunocompromized conditions (43, 55).

lesions are typically contained around the mouth and the nostrils , often originating at the commissures of the lips; lesions can likewise be seen within the buccal cavity (gums, hard palate, tongue) and, occasionally, in the oesophagus or the abomasum. In ewes, lesions are mainly observed on the teat (frequently, around the teat orifice) or the udder skin and fewer often in the inguinal area and the thigh (69).

lesions in adult animals of the disease can also be establish in the genital organs (ewes: vulva and skin-vaginal junction, rams: preputial orifice) (70) as well as in the coronet (71).

Clinical examination showed highly spread of lesions as vesicles, pustules, ulcers, and papillomatous proliferative lesions in the skin of lips ,nostrils , eyes, tail ,abdominal wall , udder and thigh regions. Figure (5-1). In lambs less than one month the lesions were more sever and associated with signs of gingivitis , hyperemic granulomatous lesions on dental pad , black scar tissue on granulomatous lesions, deformity of teeth , vesicles and pustules on lower and upper dental pad and sever bleeding area after removing of scabs .Figure (5-2) . In adult sheep, the lesions were less sever than lambs , and associated with papuloerosive lesions with a surrounding zone of hyperaemia, pustules on the lips and nose ,zone of hyperemia at the commissures of the lips and necrosis, scabs , hemorrhages ,erosive and painful area after removing of scabs from the lesions. Some of adult sheep suffering from papuloerosive lesions on the lips associated with screw worm infection .Figure (5-3). (72).



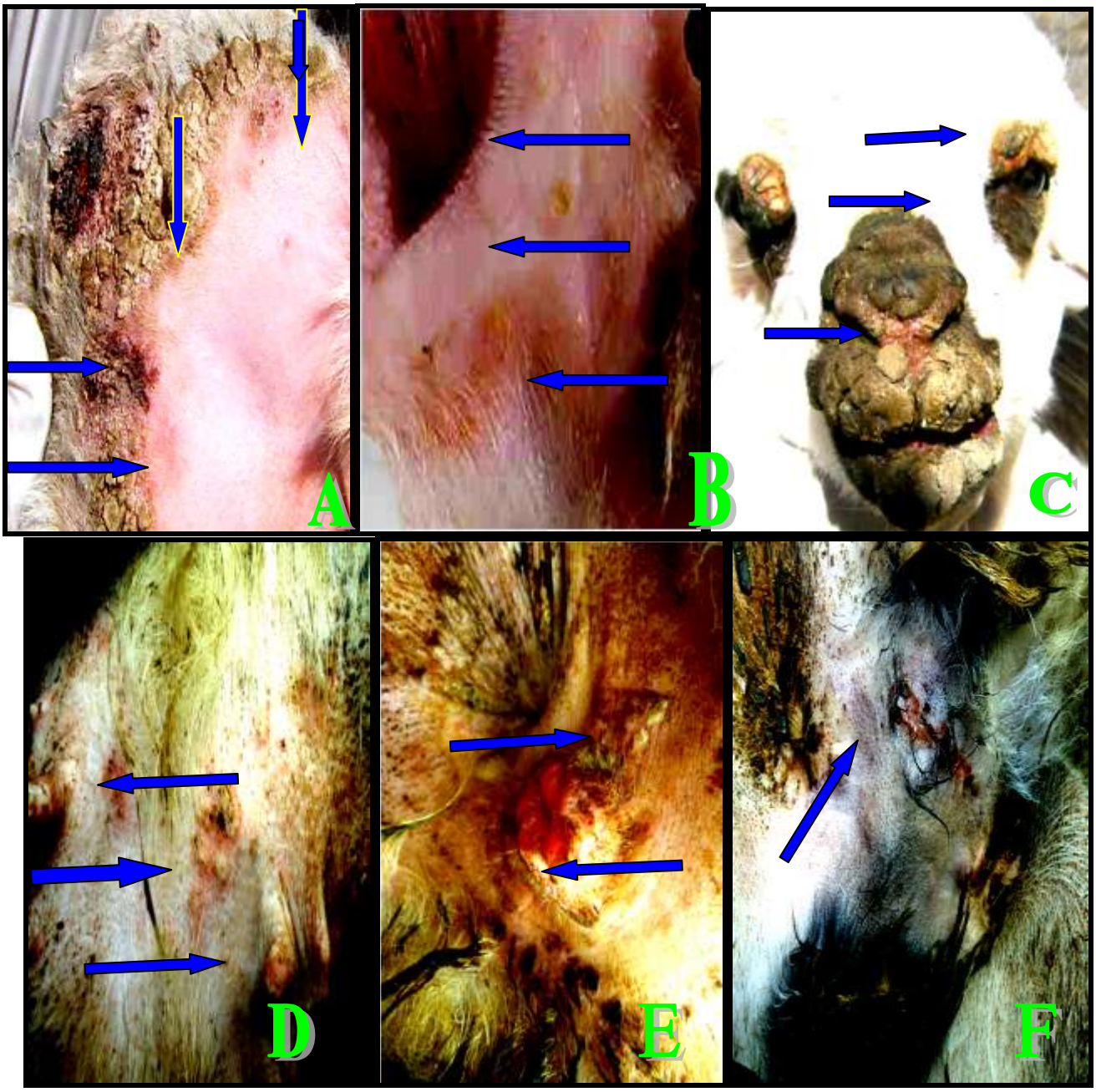


Figure (5-1):Characteristic Orf lesions spread on different regions of the skin.

(A) pustules , ulcers ,scabs and papillomatous proliferative lesions in the skin of lips ,nostrils and eyes

.(B) ulcerative lesions and scabs in the tail .

(C vesicles with erythematic zone around lesions in the thigh and inguinal regions.

(D) Nodules and vesicles in the mammary gland.

(E)vesicle and erythematic zone in the tail and vulva.

(F) laceration and ulcerative lesions with erythematic zone in the ventral abdomenal wall.

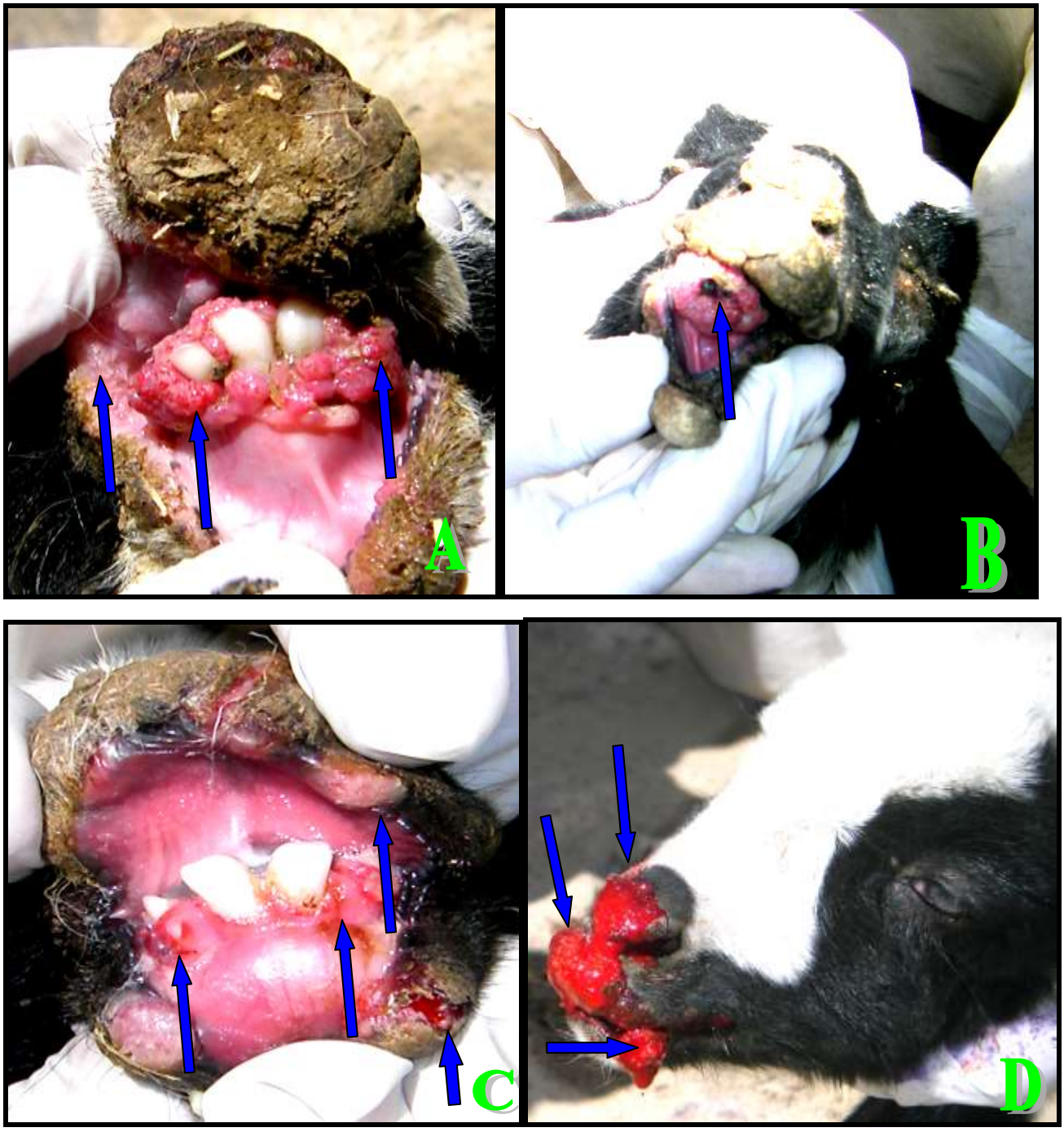


Figure (5-2):Lesions of the mouth and nose in lambs less than one month.

(A) hyperemic granulmatous lesions and deformity with separation of teeth.
(B) black scar tissue on the granulomatous lesions.
(C) vesicles on the upper and lower lips with hyperemic dental bad with loss of some incisors teeth.
(D) sever bleeding area after removing of scabs .

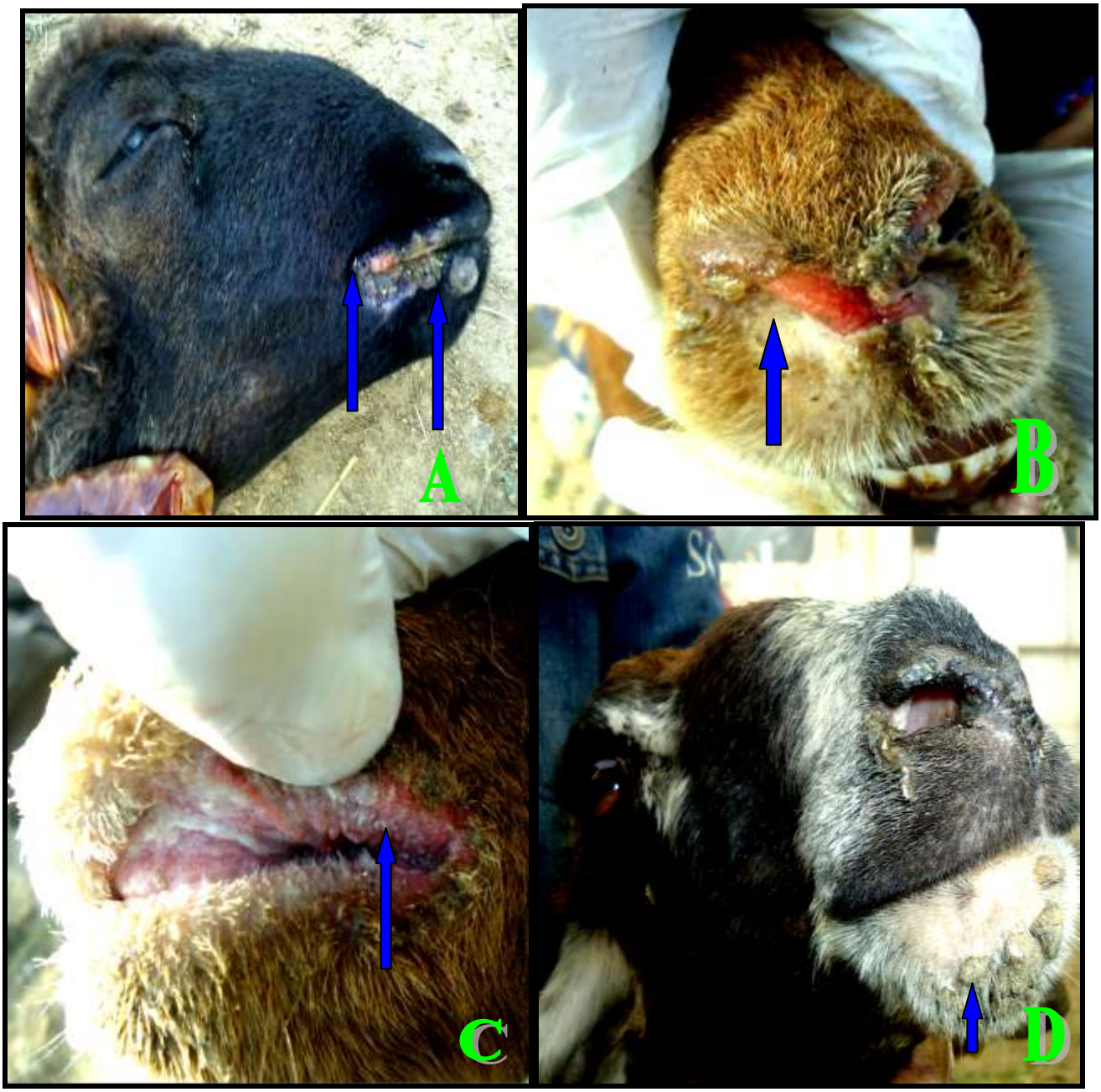


Figure (5-3): Orf lesions in adult sheep.

(A) papuloerosion with a surrounding zone of hyperaemia and pustules on the lips.

(B) necrotic , sloughed out ,erosive, hyperemic area.

(C)necrotic , slough out ,erosive, hyperemic and painful area at the commissures .

(D) pustules and dry scabs on the lower lips.

Initially , lesions progress as papules and then pustules, periods which are not usually initially experiential, and progress to elevated moderately proliferative area of granulation, and inflammation enclosed with a thick, tenacious scab. Time

development from the original lesions to the formation of scabs is about 6 to 7 days. Fresh lesions will develop throughout the first 10 days of infection . The first lesions improve at the oral mucocutaneous junction , usually at the oral commissures and are attended by swelling of the lips. From here they feast on to the muzzle and nostrils , the nearby haired skin and to a lesser amount , on to the buccal mucosa. They may seem as separate , thick scabs 0.5 cm in diameter, or coalesce and be packed local composed as a unceasing plaque. fissuring happens and the scabs are sore to the touch. They smash certainly but are problematic to eradicate from the underlying granulation. Exaggerated lambs agonize a austere setback because of limited sucking and grazing. In benevolent cases the scabs dry and drop off , and retrieval is complete in about 3 weeks (5).

lesions in young lambs are greatest frequently realized around the mouth and nostrils, often initiating at the commissures of the lips. These commonly appear as scabby lesions but , in a amount, proliferative lesions can convert infected with bacteria, which may extend the course of the disease and result in more wide involvement. Significant mortality does not occur unless the buccal cavity is also affected , when mortality can be austere. Lesions are most frequent laterally the gums, related with the erupting teeth, the hard palate and dorsum of the tongue, and may spread to involve the oesophagus. Initially , these lesions as papuloerosive and whitish with a surrounding zone of hyperaemia , hastily become necrotic and slough , and may resemble the broken vesicles of foot and- mouth disease. The subsequent ulcers on the hard palate and tongue can be up to several centimeters in diameter (14).

Severe systemic response , and delay down the alimentary tract may prime to a severe gastroenteritis, and delay down the trachea may be trailed by bronchopneumonia. Lesions may also befall in the mouth connecting the tongue, gums, dental pad or a mixture of those sites. These are additional commonly understood in outbreaks affecting lambs less than 2 months of age. In the mucosa of the mouth these lesions do

not scab but are papular erosive and enclosed by an elevated zone of hyperemia.

General painful and proliferative lesions occur on the gingival margins of the incisor teeth (73).

Physical examination in young lambs revealed that there was , fever ranged between (41 to 42C°), anorexia, depression, serous to mucopurulent nasal discharge , conjunctivitis with lacremation and hyperemic mucous membranes , some lambs suffering from yellowish to watery diarrhea , sever dehydration , unable to stand , ataxia and fall down , lambs cannot suck and scratching their mouth constantly on ground. Abnormal respiratory sounds (wheezes) with difficult inspiration dyspenea . Respiratory rate was increased and heart rate also was higher than normal .(72).

Contamination can too comprise the thigh, axilla, poll, genitalia, lower limbs and coronet, the concluding often taking the custom of verrucose masses , which are easily scraped and prone to haemorrhage . Such lesions about the coronet, sometimes denoted to as 'strawberry foot-rot', may be intensified by infection with *Dermatophilus congolensis* (46).

The venereal practice ordinarily seems quickly after the rams are twisted out, and spreads speedily within the flock. In the ewe it initiates as minor pustules at the vulva on the skin–vaginal mucosa junction , and , in the ram , at the preputial orifice. At together locations , the lesions demonstration a propensity to banquet and form shallow ulcers , which may become infected by *Fusobacterium necrophorum* generous a 'floor' of necrotic tissues to the ulcer. Exaggerated rams speedily become disinclined to mate, and the consequence can be disturbance of breeding, with a consequent lengthy lambing (74).

A malignant procedure of the disease has also been experiential in sheep. It begins with an acute incident manifested by oral vesicles, and extension of these lesions down the gastrointestinal tract , trailed later by granulomatous lesions and cracking of hooves. An uncharacteristic case of the disease in sheep after wide cutaneous thermal injury has been designated (75).

6.1. Diagnosis

Infection with Orf virus can be noticed on the basis of clinical signs but definite laboratory diagnosis is accomplished by one or a grouping of the following methods: 1) isolation and characterization of the virus in cell culture, 2) straight protest of virions, viral antigens, or viral nucleic acids and 3) finding and measurement of antibodies (76).

6.1.1. Isolation of Virus in cell culture

Virus separation in cell culture is viewed as the "gold standard" technique for detection of poxviruses. While the method does not deliver fast diagnosis of infectious agent it is important in recognition of unknown viruses, and it is the solitary method for generating stock of live virus for additional studies . Numerous primary and unremitting cell lines counting primary lamb testis , kidney , turbinate and muscle (77, 78), bovine fetal spleen and muscle (79, 80, 81), primary bovine testis and lung (82, 83), Madin-Darby ovine kidney (MDOK) (59) and Madin-Darby bovine kidney (MDBK) (84).

Cells must remained described to be apposite for cultivation of *parapoxviruses.* Cytopathic effect (CPE) is frequently seen as cell rounding , clumping and detachment, but it may revenue several sightless passages to appear (85).

Commonly, *parapoxvirus* agriculture in cell culture is observed problematic because of many ineffective attempts to isolate virus, but the details for this are unknown (86).

6.1.2. Electron Microscope

(87) carried out the diagnosis of Orf virus by electron microscopy and demonstrated the characteristic ovoid shape of the virion. Morphologically papular stomatitis virus and Orf viruse are indistinguishable (58). The most striking feature, which readily enables identification of Orf virus as well as other *parapoxviruses*, is a tubule-like structure that surround the particle in a spiral fashion (88).

(28) demonstrated the presence of 'brick shaped' particles, ~140×88 nm, presumably naked orf virus particles by electron microscopy.

(89) analyzed the skin lesion material of various ruminant species by negative staining electron microscopy, which revealed the presence of Orf virus. The virions were ovoid in shape and had crisscross pattern against an electro dense background core and were approximately 200nm long and 160nm wide.

(90) examined the tissue samples by broadcast electron microscopy and divulged the attendance of many intracytoplasmic virions in the vacuolated epithelial cells.

6.1.3. Histopathology

Orf disease causes epidermal proliferation, which is realized as dejected growths pungent the dermis . Characteristic structures include epidermal hyperplasia, hyperkeratosis, vacuolation and ballooning of keratinocytes and accumulation of scale-crust. Additionally , the occurrence of immune and inflammatory cells beneath and nearby to virus-infected cells and obvious capillary dilation and proliferation of the dermal lesions have been designated (91, 61). These virus-persuaded morphological changes can be seen with the light microscope using haematoxylin and eosin staining of thin fragments of the pretentious skin and have been secondhand in *parapoxvirus* diagnostics (89, 92, 11, 93).

In progressive parts of the lesions , abundant neutrophilic intrudes into the superficial propria and epithelium were accompanying with erosion of the upper layers of necrotic cells. Focally wide, unembellished , exuberant , perivascular to interstitial infiltration with histiocytes and lymphocytes was originate in the submucosa. It was escorted by fibroblastic proliferation and neovascularization (90).

6.1.4. Immunofluorescence assay

Immunofluorescence assays (IFA) are created on finding of virus antigen with fluorophore - labelled principal antibody (direct method) or secondary antibody (indirect IFA): the fluorophore-labelled antibody fluoresces under UV illumination

representative the attendance of virus antigen when watched with a microscope. IFA is a valuable and delicate technique since the diagnosis can be ended on the basis of only insufficient cells comprising fluorescence of the true color and expected antigen delivery . Conversely , because of this, accurate diagnosis necessitates highly skilled workers for analysis the results. In finding and characterization of *parapoxviruses,* both polyclonal improving sera from *parapoxvirus*-immunized animals and a panel of monoclonal antibodies against Orf virus have been used in indirect IFA (90).

6.1.5. Serological methods

Parapoxviruses **have been revealed to provoke obvious antibody replies in the host (80, 82, 94). The viruses are immunologically faithfully related and parade serological cross-reactivity (81, 95, 96) , while a panel of monoclonal antibodies has been revealed to be talented of selective between different *parapoxvirus* species . Various serological methods have been recycled to degree *parapoxvirus* antibodies in different animal species , but greatest of the conventional serological methods are arduous and time intense which brands them not well matched for primary diagnosis. Though , serological methods are respected in confirmatory testing and in epidemiological studies (67).**

6.1.6 . Agar gel immunodiffusion tests (AGID).

AGID is a modest process that senses *parapoxvirus* antibodies on the basis of a precipitation response between a serum sample and the virus antigen. AGID cannot

discriminate between different *parapoxvirus* species (97), nor between Orf virus and the *capripoxviruses* (98), and it is a fewer searching test than the ELISA (23). The technique has been recycled for *parapoxvirus* surveys and for validation of positive ELISA grades (99).

6.1.7. Enzyme – linked immunosorbent assay (ELISA).

These routine is fast and assists screening of large number of samples at the same time. In an ELISA test , serum samples are gestated in 96-well microtitre plates covered with purified virus antigens with antibodies being sensed with alkaline-phosphatase conjugated secondary antibodies (46), peroxidase conjugated protein A (86) or protein AG (80).

ELISA does not distinguish between changed *parapoxvirus* species either, it has been functional successfully in the conclusion of Orf virus in humans (94), camels (100) and *parapoxvirus* infections in California sea lions (101).

6.1.8. Serum neutralization tests.

The serum neutralization test events defusing antibodies in a serum sample touching a recognized titer of the test virus in cell thinking. If a fourfold escalation in the antibody titer between acute and convalescent-phase serum is pragmatic , the test is painstaking to authorize serological diagnosis. The process has been practical in finding of Orf virus in native and wild ruminants in Alaska (102), but because immunity to Orf virus infection is mainly cell-mediated and virus counteracting antibodies are usually imperceptible or advance solitary at squat level (15), the test is not dependable for primary diagnostic determinations.

6.1.9. Molecular techniques

6.1.9.1. Restriction fragment length polymorphism (RFLP).

RFLP is a way that deeds variations in genomes of uniform carefully connected virus species. In RFLP examination, the DNA is consumed with one or more constraint enzymes and the subsequent restriction fragments are unglued according to their size , by agarose gel electrophoresis, to generate different restriction fragment profiles. The RFLP examines of *parapoxvirus* genomes exposed genetic heterogeneity that has allowed species (103) and straight strain variation (104, 105).

6.1.9.2. Nucleic acid hybridization.

Nuclecic acid hybridization has been recycled together with RFLP examination to check virus classification. Digested DNA tasters are hybridized with branded probes derivative from the central and incurable regions of *parapoxvirus* genomes, and although the consequences designate that a sturdy inter-species homology occurs between counties within the central parts of the genomes, terminal sections hybridize only to the identical virus species (53).

6.1.9.3. Conventional polymerase chain reaction (PCR).

PCR is one of the greatest powerful and useful methods in virus diagnostics (106). It is grounded on the capability of thermo stable DNA polymerase to manufacture a new element of DNA during recurrent cycles of heat denaturation, annealing and extension. The mark sequence is distinct by specific oligonucleotide primers complementary to the target DNA which in crack allow the amplification of the desired province. The amount of objective DNA is doubled in each cycle subsequent in billions of facsimiles of the original sequence being twisted. The individuality of the enlarged creation can be confirmed using DNA hybridization (107) .or extra normally by direct sequencing (108, 109).

6.1.9.4. Real Time PCR (RT- PCR).

In real-time PCR (RT-PCR), the amassing of PCR creation is watched in each cycle through the PCR response by a thermocycler tool. The amplified invention can be sensed using either non-specific DNA-binding fluorogenic molecules such as SYBR Green I, or by using specific fluorogenic probes such as TaqMan® oligoprobes.

RT-PCR can be used for both qualitative and quantitative examination , it is a fast and harmless way due to small response times and because real-time discovery eradicates the essential of post-PCR dispensation; a reason that also decreases the danger of cross-corruption (106). Also , the practice of specific fluorogenic , both procedures used in the diagnosis and quantification of Orf virus or in the diagnosis of numerous *parapoxvirus* species including Orf virus , PCPV, BPSV and *sealpoxvirus* (110).

6.2. Differential diagnosis.

In record outbreaks of orf disease , the circumstances are adequately slight to cause no real anxiety about sufferers or about diagnosis. Affected epidemics of a actual severe procedure of the disease may happen , but , and are possible to be disordered with bluetongue. Precise severe cases are moreover frequently realized in contained untried sheep especially colostrum-free lambs (5).

- **Mycotic dermatitis , serous exudate formed by the skin contagion at the base of the wool fibres consequences in matting of the fibers and development of hard , thick, dry crusts in the staple. In chronic impurities , as the wool fiber raises out, the crusty exudates continue, making hard dry 'pegs' in the staple. These cannot be clear pending the wool is handled . In determined or chronic infections, the lesion may spread along the staple near the tip. In cases of self-cure, the crusty exudate parts from the skin and there is a band of normal wool underneath the crusts. Lambs in the first rare weeks of life are very predisposed to infection. Lesions are more communal done the muzzle and ears, perhaps due to close interaction with the ewe at feeding . Particular lambs can progress a general infection affecting the skin over the back and flanks. The serous exudates formula a crust over the skin , assembly it very sore if the lamb**

efforts to move. Death frequently follows as a result of secondary bacterial infections or from fly strike (111) .

- **Bluetongue , An evolution historical of four to eight days (112), is followed by fever, apathy, tachypnea, and hyperaemia of the lips and nostrils with extreme salivation and serous nasal discharge that is primarily clear, formerly becomes mucopurulent and upon drying can form a crust about the nostrils. Oedema of the tongue, lips, submandibulum and occasionally ears appears , petechiae develop on the conjunctiva and ulcers on the oral mucosa. Cyanotic tongues are start in sporadic cases. In some cases, dyspnoe, profuse haemorrhagic diarrhoea or vomiting that can cause aspiration pneumonia is noted. At the end of the pyrexia stage, pretentious sheep may have coronitis , laminitis or paresis and necrosis of striated muscles and, as a result, attitude with an arched back and are unwilling to**

move. Torticollis, dermatitis and breaks in the wool may also develop (113, 114, 115, 116).

Infection in pregnant ewes may prime to abortion , Chronically affected sheep may capitulate to other diseases such as bacterial pneumonia (117).

- **Sheep pox , the growth period varies from four to twenty-one days , but is usually one to two weeks. The disease is more severe in lambs and kids than in mature animals . Specific exact young lambs and kids may die before displaying signs of the disease. Most affected animals become feeble with no appetite. They may exhibit a high fever for a short time. Skin lesions appear as small red patches usually around the mouth , on the head , under the tail and between the legs . The centers of the patches developed depressed and turn grayish in color due to necrosis. These patches form blisters that break becoming open sores that soon progress scabs. Animals constantly have difficult breathing due to blisters inside the respiratory tract and lungs. Lesions in the mouth, nose and eyes can cause discharge and excessive salivation. Affected mucous membranes may convert necrotic and ulcerate. Nodules in the intestines can cause diarrhea.**

Depression and emaciation may be understood in some animals. Abortions may also Happen (118).

- **Ulcerative dermatitis is adequately analogous to cause misperception in diagnosis, but this disease has not been stated in many years. Facial eczema is illustrious by diffuse dermatitis and severe edema and damage to the ears. Papillomatosis (warts) requirement also to be measured in the differential diagnosis for the proliferative manifestations of contagious ecthyma , while warts are extremely surprising in sheep . (5).**
- **Foot and mouth disease** : The definitive established lesions of Orf are easily discerned from foot and mouth disease but the popular and vesicular stages seen first in the course of Orf , chiefly lesions in the mouth, can be problematic to differentiate especially when a prompt on farm differentiation is prerequisite. The elevated firm papular erosive wildlife of the lesion with the adjacent zone of hyperemia is a central separating feature in the field **(119).**

Chapter Seven……..…..Treatment & control

7. Treatment and control

There is no exact treatment . Exclusion of the scabs and the request of ointments or astringent lotions are practiced but interruption healing in most cases. The facility of soft , edible food is suggested. The combined use of diathermy debridement and cryosurgery is claimed to be effective for the proliferative intraoral lesions in young lambs (120).

In stretch of treatment and control, antibacterial may assistance fight secondary infection. In prevalent areas , suitable repellents and larvicides should be useful to the lesions. The virus is communicable to humans , and the lesions , generally limited to the hands and face , are extra proliferative and sporadically very difficult . Veterinarians and sheep handlers should employment reasonable protective precautions (121).

Quarantine of affected sucking lambs and their dams decreases the heaviness of infection , but this is seldom valuable because of its express binge . Unsoiled rams

may then be presented to the clean ewe group . Where groups are large or mixing has occurred , this practice is unlikely to be effective. During an outbreak of this form of the disease , vaccination is not optional. Vaccination may be sensible and has been reported to detention the spread in groups of young lambs. If vaccination during an outbreak is measured necessary , and profitable vaccine is not available, an autogenously vaccine can be ready and used in the flock , focus to national regulations (14).

Sheep that have improved from natural infection are extremely resilient to reinfection . Despite a multiplicity of immunogenic virus strains, the currently used marketable single-strain vaccines have produced reasonable immunity in all parts of the USA (with an infrequent exception) . Vaccine breaks seem to be due to the virulence of the infecting strain rather than to alterations in antigenicity of the vaccine. Sheep immunized touching contagious ecthyma continue susceptible to ulcerative dermatosis (122).

Vaccines should be castoff carefully to evade contaminating uninfected locations, and vaccinated animals should be isolated from unprotected stock until the scabs have

fallen off. A small amount of the vaccine is fleecy over light scarifications of the skin, usually on the privileged of the thigh. Lambs should be vaccinated when ~1 month old. For best results , a second vaccination ~2-3 months later is suggested. Non immunized lambs should be vaccinated before incoming infected feedlots. Tentative work suggests that parenteral administration of virulent vaccine induces improved immunity than does the current procedure (123).

The vaccine is set from a suspension of scabs in glycerol saline and is tinted onto a small area of scarified skin inside the thigh , or by puncturing the ear with a needle dipped in the vaccine. Vaccination is totally effective for at least 2 years, but the lambs should be examined 1 week after vaccination to insure that local responses have resulted. Deficiency of a local reaction signifies lack of viability of the vaccine or the being of a prior immunity. The immunity is not hard until 3 weeks after vaccination. A small percentage of vaccinated lambs may develop mild

lesions about the mouth because of chewing at the vaccination site. The efficiency of this vaccine is better than that of the average commercial vaccine encompassing live attenuated virus (124).

The location of vaccination is the confidential of the thigh excluding in ewes shortly before lambing , where tenacious lesions could infect the udder. In this case, the recommended site is behind the elbow. Some also advocate this site in young lambs, so that they are less likely to transfer infection to the lips through sucking the site of vaccination. The caudal fold is not recommended because of the possible enhanced risk of secondary infection. It is problematic to assess the degree or period of the immunity shaped, as a number of variables are involved , the most important being the weight of the experiment. The time selected for vaccination should be overseen by the predictable timing of the clinical disease. Where the disease does recur yearly, ewes should be vaccinated behind the elbow 6–8 weeks before lambing. The flock should be managed so that the scabs resulting from the vaccination are not shed in the lambing area, thus contributing to the pool of infectivity available to infect the lambs. Late summer outbreaks may be measured by vaccinating lambs some 6 weeks before the expected time of the appearance of the disease (14).

Chapter seven……..…..Treatment & control

Tenacity of the disease in a pastured flock from year to year is shared and in such circumstances the lambs should be vaccinated at 6-8 weeks of age. Vaccination when a few days old suggests a protective response, but pre-lambing vaccination of the ewe does not and is not recommended. Vaccination of housed lambs should be timed to avoid the usual incidence of the disease that has been experiential in previous years (125).

As a additional protective measure , elimination of harsh material from the environment is commended but is not usually feasible. For live sheep being transported from Australia to the Middle East , it is optional animals be

vaccinated well in loan of consignment to allow immunity to develop , which is maybe at least 3 weeks (58).

Because the vaccines are live virus vaccines, and shed scabs are dirty, routine vaccination alongside orf in flocks that have not knowledgeable the disease is not recommended . Outbreaks have happened from vaccine virus (5).

To date , all effective vaccines have working completely virulent virus. Future growths will depend on the isolation of distorted viruses, which absence certain genes of virulence but which retain immunogenicity. Such an attitude is currently being chased and is likely to be contingent on a detailed molecular empathetic of the virus before specific applicant viruses will become accessible (74).

Chapter Eight……Conclusions and Recommendations

8.1. Conclusions.

1. **Orf disease cause wide spread outbreaks in wide world countries .**

2. **Contagious Ecthyema cause highly economic losses due to sever lesions and high mortalities in lambs.**

3. **There were typical and characteristic clinical , histopathological and epidemiological features useful in primary diagnosis of disease .**

4. Molecular detection of the Orf virus by Real-Time (RT-PCR) and PCR consider accurate method for rapid , specific and sensitive for detection of the virus.

8.2. Recommendations .

1. **Further morphological and biological characterization of the virus by electron microscopy and isolation on tissue culture from sheep and other domestic animals.**

2. **Real-Time PCR(RT-PCR) was rapid , can be used in the evaluate of the**

viral load and in measuring the acuteness and severity according to the viral

3. Estimation of blood antibodies of Sheep flocks vaccinated with sheep pox virus and comparative these antibodies levels of the same flocks associated with highly occurrence outbreaks of the Orf virus infection .

4- Isolation and promotion of the Orf virus for manufacture alive attenuated vaccine .

References……...

1- Gokce, H.I.; Genc, O. and Gokce, G. (2005).Sero-prevalence of Contagious Ecthyma in Lambs and Humans in Kars, Turkey.*Turk. J. Vet. Anim. Sci., 29: 95-101.*

2- Hosamani, M. ; Bhanuprakash, V. ; Scagliarini, A. and Singh, R.K.(2006). Comparative sequence analysis of major envelope protein gene (B2L) of Indian orf viruses isolated from sheep and goats. *Vet. Microbiol. 116, 317-324.*

3- Gallina, L.; Scagliarini, L. ; McInnes, C. J. ; Guercio, A.; Purpari, G.; Prosperi, S. and Scagliarini, A. **(2008).** Parapoxvirus in goats: experimental infection and genomic analysis. ***Veterinary Research Communica-tions, 32, S203–S205.***

4- Tryland, M. ; Klein, J. ; . Nordoy , E. S. and. Blix, A. S. (2005). Isolation and partial characterization of a parapoxvirus isolated from a skin lesion of a Weddell seal. *Virus Re-search, 108, 83–87.*

5- Radostitis,O.M ; Gay , C.C. ; Hinchcliff,K.W. and Constable , P.D.(2007).veterinary medicine , 10th Edition.London : W.B.Saunders Company Ltd,P.1412- 1420.

6- Hawayek, LH. and Rubeiz, N. (2007). Orf [online]. eMedicine; Available at: *http://www.emedicine.com/derm/ topic605.htm.* .

7- Giadinis, N. D. ; Filliusis, G. ; Lafi, S. Q. ; Panousis, N.; Pourliotis, K. ; Bojkovski, J. and Karatzias, H. (2007). *Vet. glasnik 61 (5-6) 301 – 312* .

8- Tucson, AZ. (2003). Farm animal zoonotic and reportable diseases. *VSC/443/543 Research Animal Methods.* Class notes for: November 7,03.

9- Lewis, C. (1996).Update on orf. *In Farm Animal Practice, 18: 376-381.*

10- de la Concha-Bermejillo, A. ; Guo, Z. Zhang, J. and Waldron D.(2003). Severe persistent orf in young goats . *J Vet Diagn Invest 15:423–431*.

11- Nollens, H.H. ; Jacobson, E.R. ; Gulland, F.M. ; Beusse, D.O. ; Bossart, G.D. ; Hernandez, J.A. ; Klein, P.A. and Condit, R.C.**(2006b).** Pathology and preliminary characterization of a parapoxvirus isolated from a California sea lion (Zalophus californianus). ***J. Wildl. Dis. 42, 23-32.***

12- Coates, J.W. and Hoff, S. (1990). Contagious ecthyma: An unusual distribution of lesions in goats. *Canadian Veterinary Journal, 31: 209-210.*

References.....

13- Mercer, A. ; Fleming, S.; Robinson, A. ; Nettleton, P. and Reid, H. **(1997)**: Molecular genetic analyses of parapoxviruses pathogenic for humans. ***Archives of Virology Supplementum 13, 25-34.***

14- Reid, HW. and Rodger, SM. (2007). Orf. In: Diseases of Sheep.. 4 edition. Edited by: *Aitken AI. Oxford: Blackwell;:297-306.*

15- Haig, D.M. and Mercer, A.A. (1998). Ovine diseases. Orf. *Vet. Res. 29, 311-326*.

16- Jae-Ku, Oem. ; In-Soon, Roh. ; Kyung-Hyun, Lee. ; Kyoung-Ki, Lee. ; Hye-Ryoung, Kim. ; Young-Hwa, Jean. and O-Soo, Lee. **(2009)** .:Phylogenetic analysis and characterization of Korean orf virus from dairy goats: ***case report, virology journal , 16 octobre.***

17- Mondal, B . ; Bear, A.K. ; Hosamani, M .; Tembhurne ,P.A. and Bandyopadhyay, S.K. (2006). Detection of orf virus from an outbreak in goats and its genetic relation with other parapoxviruses .*Vet Res Comm,30, 531 – 539*

18- Hosamani, M .; Scagiliarini, A.; Bhanuprakash ,V .; Mclnnes, C.J. and Singh, R.K.(2009). orf: an update on current research and future perspectives . *Expert Rev Anti Infect Ther , 7,879-893.*

19- Oxford English Dictionary. Oxford (UK): *Oxford University Press; 2012.*

20- Webster's Third New International Dictionary (1969). Unabridged. Springfield (MA): G & C Mirriam Co.

21- Stedman's Medical Dictionary. 27th ed. Baltimore: Lippincott Williams & Wilkins; (2000).

22- Al-Nadda , K.S.(1983). Isolation and characterization of Orf virus. A thesis Submitted to the Council of the College of Veterinary Medicine, Baghdad University. (In Arabic)

23- Housawi , F.M.T. ; Abu-Elzien , E.M.E. ; Al Afaleq , A.I. and Amin, M.M.(1992). Sero-surveillance for Orf Antibodies in sheep and goats in Saudi Arabia ,Empolying the ELISA Technique .*J.comp.path., Vol.106: 153-158.*

24- Moss, B.(2001). *Poxviridae*: The viruses and their replication. In: D.M. Knipe, P.M. Howley, D.E. Griffin, R.A. Lamb, M.A. Martin, B. Roizman, S.E. Straus (Eds.), Fundamental virology. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 1249-1283.

References……...

25- Thomas, V.; Flores, L. and Holowczak, J.A.(1980). Biochemical and electron microscopic studies of the replication and composition of milker's node virus. *J. Virol. 34, 244-255.*

26- Radostits ,O. M. ; Blood,D. C . and Gray, C. C. (1994). (eds) *Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses* (8th edn). Baillière, London.

27- Ali, M.H. ; Ahmed, M.H. ; Tamam, S.M. ; Arafa, A. ; Saad, A.A. ; Ali, W.F. and Madbouly, H.M.(2013). Molecular Characterization of Orf Virus Isolated from Sheep and Goats in Egypt. *Global Veterinaria 11 (1): 98-106* .

28- Torfason, E.G. and Guonadottir, S. (2002). Polymerase chain reaction for laboratory diagnosis of orf virus infections. *Journal of Clinical Virology, 24: 79-84.*

29- Reid.H.W.(2000).Orf in :Disease of sheep .(Martin,W.B.and Aitken I.D.),3th ed., Marston Book Services Ltd., P.261-265.

30- Shaheen , M.G.(1992). Study of isolates epidemic contagious ecthyma virus and comparative with live attenuated vaccine of local isolated of Hulileh strain. A thesis Submitted to the Council of the College of Veterinary Medicine, Baghdad University. (In Arabic).

31-Davis,B.D.;Dublecco,R.;Elisen,H.N.;Ginsberg,H.S.andWood,W.D.(1990).Microbiology, 4th ed . Harper & Row.Publishers.P.948 – 959.

32- Munz ,E. ; Gurtner ,T. and Huebschle, O. J. B. (1991). Zur Orf-Infection bei Boerenziegen in Namibia. *Tierärztliche Umschau 46: 86–93*

33- Valder ,W. A. ; Straub, O. C. ; Thiel, W. ; Wacheörfer, G. and Zettl, K. (1979). Ecthyma Contagiosum des Schafes-Wandel des klinischen Bildes. *Tierärztliche Umschau 34: 824–836.*

34- Couch and Alan John; (1983). "The Development and Host Response to Ovine Contagious Pustular Dermatitis". A thesis submitted to the Faculty of Science of the University of Clark, R.H., Kenyon, J.C., Bartlett, N.W., Tscharke, D.C., Smith, G.L., 2006. Deletion of gene A41L enhances vaccinia virus immunogenicity and vaccine efficacy. *J. Gen. Virol. 87, 29-38. of New England*, Armidale, N.S.W., in partial fulfilment of the requirements of the degree of Bachelor of Science with Honours.

35- Zeljko, Cac. ; Ana, Beck. ; Tomislav, Bedekovic. ; Zeljko, Cvetnic. And Branko, Sostaric. (2010). Phylogenetic analysis of Croatian orf viruses isolated from sheep and goats Ivana Lojkic1. *Virology Journal, 7:314.*

References.....

36- Lederman, E.R.; Austin, C.; Trevino, I.; Reynoldsm,G.; Swanson,H.; Cherry, B.; Ragsdale, J.; Dunn, J.; Meidl, S.; Zhao, H.; Li, Y.; Pue, H. and Damon, I.K. (2007).Orf virus infection in children: clinical characteristics, transmission, diagnostic methods, and future therapeutics. *Pediatr. Infect. Dis. J. 26, 740-744.*

37- Gumbrell, R.C. and Mcgregor, D.A. (1997). Outbreak of severe fatal orf in lambs. *Vet Rec. 141, 150-151.*

38- Unal, G., Gundes, S. and Ustun, M. (2002). Human orf: Echtyma contagiosum report of five cases. *Turk. J. Med. Sci. 32, 173-175.*

39- de la Concha-Bermejillo, A.; Anderson, NV. And Bretzlaf K, *et al*.,(1998). Overview of diseases and drug needs for sheep and goats. Veterinarians' and producers' perspectives. *Vet Human Toxicol 40 Suppl 1:7–12.]*

40- Allworth, MB. ; Hughes, KL. And Studdert, MJ.(1987). Contagious pustular dermatitis (orf) of sheep affecting the ear following ear tagging. *Aust Vet J 64:61–62.*

41- Ames ,T.R. ; Robinson, R.A. ; O'Leary, T.P. and Fahrmann, J.W.(1984). Tail lesions of contagious ecthyma associated with docking J. Am. Vet. Med. Assoc ; 184 : 88.

42- Stephen, M. Apatow. (2010):One Medicine: One Health (Zoonotic Disease) Online Course, Biodefense Reference Library Foreign Animal and Zoonotic Disease Center.

43- Steinhart, B. (2005). Orf in humans: dramatic but benign. *CJEM. 7, 417-419.*

44- McKeever, D.J. and Reid, H.W. (1986). Survival of orf virus under British winter conditions. Veterinary Record, 118, 613–614.

45- Yeruham, I. ; Perl, S. and Abraham, A. (2000). Orf infection in four sheep flocks. *Vet J 160:74–76.*

46- McKeever, D.J. (1986). Studies of the immunology and epidemiology of orf. *PhD thesis,Edinburgh University.*

47-Gukce, H.Ü. and Woldehiwet, Z. (1999).Ehrlichia (Cytoecetes) phagocytophila predisposes to severe contagious ecthyma (orf) in lambs. J. Comp. *Pathol., 121: 227-240.*

48- Ndikuwera, J. ; Odiawo, G.O. ; Usenik, E.A. ; Kock, N.D. ; Ogaa, J.S. and Kuiper, R. (1992). Chronic contagious ecthyma and caseous lymphadenitis in two Boer goats. *Vet. Rec., 131: 584-585.*

References……...

49- Reid, H.W. (1991)Orf. In: Martin, W.B., Aitken, I.D., Eds. Diseases of Sheep. 2nd ed., Blackwell Scientific Publications,Oxford, pp: 265-269.

50- Nettleton, P.F. ; Gilray, J.A. ; Yirrell, D.L. ; Scott, G.R. and Reid, H.W. (1996b). Natural transmission of orf virus from clinically normal ewes to orf-naive sheep. *Vet. Rec., 139: 364-366.*

51- Gul, Y.(2006). Infectious diseases. *In*: Internal Medicine of Ruminants(Cattle, Sheep-Goats), GUL Y. (ed.), 2nd edition, Medipres Matbaacılık Yayıncılık Ltd. Şti, Malatya. pp.: 129-165.

52- Reid, H.W. **(2003).** Orf. *In*: Disease of Sheep, 3rd edition, Martin, W.B. ; Aitken I.D. (eds.), ***Science, Blackwell., pp: 261-266.***

53- Robinson, A.J. and Mercer, A.A.(1995). Parapoxvirus of red deer: evidence for its inclusion as a new member in the genus parapoxvirus. ***Virology 208, 812–815.***

54- Haig, D.M. and McInnes, C.J. (2002). Immunity and counter-immunity during infection with the parapoxvirus orf virus. ***Virus Research, 88: 3-16.***

55- Geerinck, K.; Lukito,G.; Snoeck,R.;Devos, R. and Declercq, E. (2001). (VANRENTERGHEM Y., DEGREEF H., MAES B. A case of human orf in an immunocompromised patient treated successfully with cidofovir cream. ***J. Med. Virol. 64, 543-549.***

56- Tom, W. and Sheila, F. (2008). Poxvirus infections. Fitzpatrick's dermatology in general medicine. 7th edition. Wolf K., Goldsmith L.A., Katz S.I., Gilchrest B.A., Paller A.S., Leffell D.J. New York: The McGraw-Hill Companies, Inc: 1899-1913.

57- Murphy, F.A. ; Gibbs, E.P.J. ; Horzinek, M.C. and Studdert, M.J. (1999). Poxviridae. Veterinary virology. 3rd ed.: ***Academic Press, San Diego, pp. 277-291.***

58- Robinson, A.J. and Balassu, T.C. (1981). Contagious pustular dermatitis (orf). ***The Veterinary Bulletin, 51: 771-782.***

59- Guo, J.; Zhang, Z.; Edwards, J. F. ; Ermel, R. W. ; Taylor, C. and de la Concha-Bermejillo, A. (2003). Characterization of a North American orf virus isolated from a goat with persistent, proliferative derma-titis. ***Virus Research, 93, 169–179.***

60- Abu Elzein, E.M.E. and Housawi , F.M.T.(1997) . Sever long lasting contagious ecthyma infection in agoat's kid.*J.Vet.Med .B 44: 561-564.*

61- Mckeever, D.J. ; Jenkinson, D.M. ; Hutchinson, G. and Reid, H.W. (1988). Studies of the pathogenesis of orf virus infectioin in sheep. ***Journal of Comparative pathology. 99 : 317 – 328.***

References……...

62- Jenkinson, D.M. ; Hutchison, G. ; Onwuka, S.K. and Reid, H.W. (1991). Changes in the MHC class II_ dendritic cell population of ovine skin in response to orf virus infection. ***Veterinary Dermatology, 2: 1-9.***

63- Lear, A. ; Hutchinson, G. ; Reid, H.W. ; Norval, M. and Haig, D.M. (1996). Phenotypic characterisation of the dendritic cells accumulating in ovine dermis following primary and secondary orf virus infections. ***European Journal of Dermatology, 6: 135-140.***

64- Haig, D.M. ; McInnes, C.J. ; Deane, D. ; Lear, A. ; Myatt, N. ; Reid, H.W. ; Rothel, J. ; Seow, H.F. ; Wood, P. ; Lyttle, D. and Mercer A.A. (1996a). Cytokines and their inhibitors in orf virus infection. ***Veterinary Immunology and Immunopathology, 54: 261-267.***

65- Haig, D.M.; Hutchison, G. ; Thomson, J. ; Yirrell, D. and Reid, H.W.(1996b). Cytolytic activity and associated serine protease expression by skin and afferent lymph CD8+ T cells during orf virus reinfection. ***Journal of General Virology, 77: 953-961.***

66- Haig, D.M. ; Deane, D. ; Percival, A. ; Myatt, N. ; Thomson, J. ; Inglis, L. ; Rothel, J. ; Seow, H.F. ; Wood, P. ; Millar, H.R.P. and Reid, H.W. (1996c). The cytokine response of afferent lymph following orf virus reinfection of sheep. ***Veterinary Dermatology, 7: 11-20.***

67- Housawi, F.M.; Roberts, G.M. ; Gilray, J.A. ; Pow, I. ; Reid, H.W. ; Nettleton, P.F. ; Sumption, K.J. ; Hibma, M.H. and Mercer, A.A.(1998). The reactivity of monoclonal antibodies against orf virus with other parapoxviruses and the identification of a 39 kDa immunodominant protein. ***Arch. Virol. 143, 2289-2303.***

68- Fenner ,F.; Bachmann, PA.; Gibbs, EPJ.; Murphy, FA.; Studdert, MJ.and White, DO. (1987). Veterinary virology. San Diego, CA: ***Academic Press Inc.Orf; p. 397.***

69- Nandi, S. ; De Ujjwal, K. and Chowdhury, S.(2011). Current status of contagious ecthyma or orf disease in goat and sheep -A global perspective. ***Small Rumin Res , 96:73-82.***

70- Gouletsou, PG. and Fthenakis, GC.(2010). Clinical evaluation of reproductive ability of rams. ***Small Rumin Res, 92:45-51.***

71- Egerton, JR.(2007). Diseases of the feet. ***In Diseases of Sheep.. 4 edition. Edited by: Aitken AI. Oxford: Blackwell,:273-281.***

References……...

72- Salah,M.Karim(2015). Study of some clinical and epidemiological features and rapid detection of Orf disease in sheep by molecular techniques. A Thesis

73- Hawkins, C.D. ; Ellis, T.M. ; Davies, M.K. ; Peet, R.L. and Arkinson, J.P. (1991): An unusual outbreak of contagious ovine ecthyma. ***Australian Veterinary Journal, 68: 210-211.***

74- Nettleton, P.F. ; Brebner, J. and Pow, I. ***et al.*** **(1996a) .Tissue culture-propagated orf virus vaccine protects lambs from orf virus challenge.** ***Veterinary Record, 138, 184–6.***

75- Hooser ,SB. ; Griesemer, R.A. and Cole, C.R. (1989). Bovine papular stomatitis. II. The experimentally produced disease. ***Am. J. Vet. Res. 22, 473-481.***

76- Barthold, S.W.; Bowen, R.A.; Hedrick, R.P.; Knowles, P.; Lairmore, M.D.; Parrish, C.R.; Saif, L.J.and Swayne, D.E.(2011). ***Poxviridae*****. In: N.J. MacLachlan, E.J. Dubovi (Eds.), Fenner's Veterinary Virology.** ***Elsevier Inc., London, UK, pp. 151-165.***

77- Delhon, G.; Tulman, E.R.; Afonso, C.L.; Lu, Z.; Concha-Bermejillo, A.; Lehmkuhl, H.D.; Piccone, M.E.; Kutish, G.F. and Rock, D.L. (2004). Genomes of the parapoxviruses orf virus and bovine Papular stomatitis virus. ***J. Virol. 78, 168-177.***

78- Kottaridi, C.; Nomikou, K. ; Lelli, R. ; Markoulatos, P. and Mangana, O. **(2006a).** Laboratory diagnosis of contagious ecthyma: comparison of different PCR protocols with virus isolation in cell culture. ***J. Virol. Methods 134, 119-124.***

79- Hessami, M.; Keney, D.A.; Pearson, L.D. and Storz, J. (1979). Isolation of parapox viruses from man and animals: cultivation and cellular changes in bovine fetal spleen cells. ***Comp.Immunol. Microbiol. Infect. Dis. 2, 1-7.***

80- Inoshima, Y. ; Shimizu, S. ; Minamoto, N. ; Hirai, K. and Sentsui, H. (1999). Use of protein AG in an enzyme-linked immunosorbent assay for screening for antibodies against parapoxvirus in wild animals in Japan. ***Clin. Diagn. Lab. Immunol. 6, 388-391.***

81- Lard, S.L.; Roehrig, J.T. and Pearson, L.D.(1991). Differentiation of parapoxviruses by application of orf virus-specific monoclonal antibodies against cell surface proteins. ***Vet. Immunol. Immunopathol. 28, 247-258.***

References……...

82- Kuroda, Y. ; Yoshida, M. ; Shibahara, T. ; Matsui, T. ; Nakane, T. ; Hara, H. ; Inoshima, Y. and Sentsui, H. (1999). An epidemic of parapoxvirus

infection among cattle: isolation and antibody survey. ***J. Vet. Med. Sci. 61, 749-753.***

83- Mercer, A.A. ; Yirrell, D.L. ; Reid, H.W. and Robinson, A.J. (1994). Lack of cross-protection between vaccinia virus and orf virus in hysterectomy-procured, barrier-maintained lambs. ***Vet. Microbiol. 41, 373-382.***

84- Klein, J. and Tryland, M. (2005). Characterisation of parapoxviruses isolated from Norwegian semi-domesticated reindeer (Rangifer tarandus tarandus). ***Virol. J. 2, 79.***

85- Dal Pozzo, F., Martinelle, L., Gallina, L., Mast, J., Sarradin, P., Thiry, E., Scagliarini, A., Buttner, M. and Saegerman, C.(2011). Original findings associated with two cases of bovine papular stomatitis. ***J. Clin. Microbiol. 49, 4397-4400.***

86- Suzuki, T. ; Minamoto, N. ; Sugiyama, M. ; Kinjo, T. ; Suzuki, Y. ; Sugimura, M. and Atoji, Y.(1993). Isolation and antibody prevalence of a parapoxvirus in wild Japanese serows (Capricornis crispus). ***J. Wildl. Dis. 29, 384-389.***

87- Wilson, T.M. and Sweeny, P.R. (1970). Morphological studies of seal poxvirus. ***Journal of Wildlife Diseases, 6: 94-97.***

88- Spehner, D. ; De Carlo, S. ; Drillien, R. ; Weiland, F. ; Mildner, K. ; Hanau, D. and Rziha, H.J. (2004). Appearance of the Bona Fide Spiral Tubule of Orf Virus is Dependent on an Intact 10-Kilodalton Viral Protein. ***Journal of Virology, 79:15: 8085-8093.***

89- Guo, J.; Rasmussen, J.; Wünschmann, A. and de la Concha-Bermejillo, A.(2004). Genetic characterization of orf viruses isolated from various ruminant species of a zoo. ***Veterinary Microbiology, 99: 81-92.***

90- Kanou,Y. ; asuo Inoshima, Y. ; Shibahara,T. ; Ishikawa, Y. ; Kadota, K. ; Ohashi,S. ; Morioka, K. ; Yoshida, K. and Yamada, S . (2005). Isolation and Characterization of a Parapoxvirus from Sheep with Papular Stomatitis. ***JARQ 39 (3), 197 – 203.***

91- Groves, R.W.; Wilson-Jones, E. and MacDonald, D.M.(1991). Human orf and milkers' nodule: a clinicopathologic study. ***J. Am. Acad. Dermatol. 25, 706-711.***

92- Leonard, D.; Otter, A.; Everest, D.; Wood, A.; McInnes, C. and Schock, A.(2009). Unusual bovine papular stomatitis virus infection in a British dairy cow. ***Vet. Rec. 164, 65.***

93- Vikoren, T. ; Lillehaug, A. ; Akerstedt, J. ; Bretten, T. ; Haugum, M. and Tryland, M.(2008). A severe outbreak of contagious ecthyma (orf) in a free-ranging musk ox (Ovibos moschatus) population in Norway. ***Vet. Microbiol. 127, 10-20.***

94- Yirrell, D.L. ; Reid, H.W. ; Norval, M. and Howie, S.E.(1989). Immune response of lambs to experimental infection with Orf virus. ***Vet. Immunol. Immunopathol. 22, 321-332.***

Reed, D.E.(1983). Reaction of convalescent bovine 95- Rosenbusch, R.F.and antisera with strainspecific antigens of parapoxviruses. ***Am. J. Vet. Res. 44, 875-878.***

96- Wittek, R. ; Herlyn, M. ; Schumperli, D. ; Bachmann, P.A. ; Mayr, A. and Wyler, R.(1980). Genetic and antigenic heterogeneity of different parapoxvirus strains. ***Intervirology 13, 33-41.***

97- Papadopoulos, O.A. ; Dawson, P.S. ; Huck, R.A. and Stuart, P.(1968). Agar gel diffusion studies of paravaccinia viruses. ***J. Comp. Pathol. 78, 219-225.***

98- Chand, P. ; Kitching, R.P. and Black, D.N.(1994). Western blot analysis of virus-specific antibody responses for capripox and contagious pustular dermatitis viral infections in sheep. ***Epidemiol. Infect. 113, 377-385.***

99- Inoshima, Y.; Yamamoto, Y.; Takahashi, T.; Shino, M. ; Katsumi, A. ; Shimizu, S. and Sentsui, H. (2001a). Serological survey of parapoxvirus infection in wild ruminants in Japan in 1996-9. ***Epidemiol. Infect. 126, 153-156.***

100-Azawi,S.M., Carter , S.D.and Woldehiwet ,Z.(1995). Immune resposes of the camel (camelus dromedarius) to contagious ecthyma (Orf) virus infection. ***Vet. Microbiol. 47, 119-131.***

101- Nollens, H.H. ; Gulland, F.M. ; Hernandez, J.A. ; Condit, R.C. ; Klein, P.A. ; Walsh, M.T. and Jacobson, E.R.(2006a). Seroepidemiology of parapoxvirus infections in captive and free-ranging California sea lions Zalophus californianus. ***Dis. Aquat. Organ. 69, 153- 161.***

102- Zarnke, R.L. ; Dieterich, R.A. ; Neiland, K.A. and Ranglack, G.(1983). Serologic and experimental investigations of contagious ecthyma in Alaska. ***J. Wildl. Dis. 19, 170-174***

103- Gassmann, U.; Wyler, R. and Wittek, R.(1985). Analysis of parapoxvirus genomes. ***Arch. Virol.83, 17-31.***

104- Rafii, F. and Burger, D.(1985). Comparison of contagious ecthyma virus genomes by restriction endonucleases. ***Arch. Virol. 84, 283-289.***

References……...

105- Robinson, A. J. (1992). In vivo recognition of orf virus early transcriptional promoters in a vaccinia virus recombinant. ***Virology 187, 464–471.***

106- Mackay, I.M. ; Arden, K.E. and Nitsche, A. (2002). Real-time PCR in virology. ***Nucleic Acids Res. 30, 1292-1305.***

107- Inoshima, Y.; Morooka, A. and Sentsui, H. (2000). Detection and diagnosis of parapoxvirus by the polymerase chain reaction. ***J. Virol. Methods 84, 201-208.***

108- Inoshima, Y.; Murakami, K.;Yokoyama, T. and Sentsui, H. **(2001).** Genetic heterogeneity among parapoxviruses isolated from sheep, cattle and Japanese serows (Capricornis crispus). ***J. Gen. Virol. 82, 1215-1220.***

109- Lojkic, I.; Cac, Z.; Beck, A.; Bedekovic, T.; Cvetnic, Z. and Sostaric, B. (2010). Phylogenetic analysis of Croatian orf viruses isolated from sheep and goats. ***Virol. J. 7, 314.***

110- Nitsche, A.; Buttner, M. ; Wilhelm, S. ; Pauli, G. and Meyer, H.(2006). Real-time PCR detection of parapoxvirus DNA. ***Clin. Chem. 52, 316-319.***

111- Edwards, J.R. (1988). Ovine dermatophilosis. In: ***Proceedings 110 of Post Graduate Committee in Veterinary Science,*** **University of Sydney,** ***Sheep Health and Production Refresher Course, pp. 383–96.***

112- Tweedle, N. and Mellor, PS. (2002).Technical review – bluetongue: The virus, hosts and vectors. Version 1.5. Report to the Department of Health, ***Social Services and Pub lic Safety U.K. (DEFRA), 25 p.***

113- Anonymous. (2004): Manual of diagnostic test and vaccines for terrestrial animals. 5th ed. Chapter 2.1.9. Bluetongue, ***Office International Des Epizooties, Paris.***

114- Darpel, KE. ; Batten, CA. ; Veronesi, E. ; Shaw, AE . ; Anthony, S.; Bachanek-Bankowska, K.; Kgosana, L.; Bin-Tarif, A.; Carpenter, S.; Muller-Doblies, UU.; Takamatsu, H-H.; Mellor, PS.; Mertens, PPC. and Oura, CAL. (2007). Clinical signs and pathology shown by British sheep and cattle infected with bluetongue virus serotype 8 derived from the 2006 outbreak in northern Europe. ***Veterinary Record 161, 253–261.***

115- Elbers, ARW.; Backx, A.; Meroc, E.; Gerbier, G.; Staubach, C.; Hendrickx, G.; van der Spek, A.and Mintiens, K. (2008). Field observations during the bluetongue serotype 8 epidemic in 2006 I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium, France and the Netherlands. *Preventive Veterinary Medicine 87, 21–30.*

References……...

116- Kirschvink ,N. ; Raes, M. ; Saegerman, C. (2009).: Impact of a natural bluetongue serotype 8 infection on semen quality of Belgian rams in 2007. *Veterinary Journal 182, 244–251.*

117- MacLachlan, NJ. and Gard, G. (2009). Clinical signs and pathology. In: Mellor P, Baylis M, Mertens PPC (eds.): Bluetongue. Academic Press, London. 285–293.

118- Spickler, A. R. and Roth, J.A. (2006). Emerging and Exotic Diseases of Animals, 3rd ed. Iowa State University, Ames, IA, USA.

119- Watson,P. (2004). Differential diagnosis of oral lesions and FMD in sheep. *Inpract.26.4.182 Clinical Practice Farm Animal Practice*

120- Meynink, SE. ; Jackson, PG. and Platt, D. (1987). Treatment of intra oral orf lesions in lambs using diathermy and cryosurgery. *Vet Rec 121:594.*

121- Kuhl, JT. ; Huerter, CJ. And Hashish, H.(2003). A case of human orf contracted from a deer. *Cutis.71:288-290.*

122- Acha,P.N. and Szyfres,B. (2003).Pan American Health Organization2. [PAHO]).Zoonoses and communicable diseases common to man and animals. Volume Chlamydiosis, rickettsioses, and viroses. 3rd ed. Washington DC: PAHO, *Scientific and Technical Publication No. 580. Contagious ecthyma; p. 80-83.*

123- Centers for Disease Control and Prevention .(2006). Orf virus in-fection in humans--New York, Illinois, California, and Tennessee, 2004-2005. *Morb Mortal Wkly Rep. 2006;55:65-68.(CDC).*

124-Gilray, J.A. ; Nettleton, P.F. and Pow, I., *et a1*. (1998). Restriction endonuclease profile of orf virus isolates from the British Isles. *Vet Rec ; 143:237- 240.*

125-Buddie, B. M. and Pulford, H. D.(1984). Effect of passively-acquired antibodies and vaccination of the immune response to contagious ecthyma virus *.Vet. Microbiol., 9, 515* .