

Molecular and Clinical investigation of lumpy skin disease in cattle in AL .Qadissiya province

Khalefa Ali Mansour Hassan HachimNaser Asaad jassim

Department of Internal Medicine, College of Veterinary Medicine,
University of Al-Qadissiya
E. mail: Khalefa.mansour@yahoo.com

Abstract

In the present study describes the clinical, histological, and molecular diagnosis of lumpy skin disease (LSD) which prevalence as Outbreak in AL -Qadissiya province. Sixteen cases suspected infection with (LSD) in cattle depending on the clinical signes which appear clearly in diferent area in AL -Qadissiya province. All suspected animals were clinically examined and a tentative diagnosis of LSD was done upon noticing of typical clinical signs . Specimens (skin biopsies) were collected from nodular skin lesions of infected animals for histopathological examination then the PCR assay was performed to confirm the found of disease in the suspected cases . The result of The physical and clinical examination revealed atypical clinical manifestation of (LSD) .histopathological result showed characteristic pathological change. The results of PCR (conventional PCR)technique for detection of specific primer P23 antigen gene (720bp)of(LSD)virus showed 14 out of 16 were positive result from collected samples .

In conclusion the lumpy skin disease is wide spread in a AL -Qadissiya province and the disease have a typical and characteristic clinical ,histopathological features that used in tentative diagnosis .PCR technique was rapid ,sensitive and specific for detection of the (LSD)virus and confirmative diagnosis of disease .

Key word: lumpy skin disease, molecular, cattle, histopathology, PCR.

التحري الجزيني والسريري لمرض تكتل الجلد العقيدي في الابقار في محافظة القادسية

اسعد جاسم

حسن حاجم ناصر

خليفة علي منصور

الخلاصة:

تم في هذه الدراسة وصف للتشخيص السريري، النسيجي والجزيئي لمرض تكتل الجلد العقيدي والمنتشر كثرة مرض في محافظة الديوانية. جميع الحيوانات المتوقعة اصابتها فحصت سريريا كتشخيص اولي للمرض بالاعتماد على العلامات السريرية النموذجية. تم جمع عينات الجلد (خزعات جلدية) من العقيدات المنتشرة على جسم الحيوان المصاب ثم فحصت نسيجيا وتشخيص باستخدام تقنية تفاعل البلمرة المتسلسل لتأكيد التشخيص. اظهرت نتائج الفحص السريري والنسيجي وجود العلامات السريرية والتغيرات النسيجية المميزة للمرض كذلك اظهرت نتائج تفاعل البلمرة المتسلسل (14) حالة مصابة من بين (16) حالة مشتبه بالإصابة باستخدام البادئ الخاص p32 ذات الطول الموجي (720bp) الخاص بفيروس مرض تكتل الجلد العقيدي. نستنتج من هذه الدراسة ان مرض تكتل الجلد العقيدي منتشر بمحافظة الديوانية وله علامات سريرية ونسجية نموذجية واضحة استخدمت كطريقة للتشخيص الاول للمرض كذلك تقنية تفاعل البلمرة المتسلسل. الكلمات المفتاحية/ مرض تكتل الجلد العقيدي/جزيئي/ الابقار /النسجية المرضية /تفاعل البلمرة المتسلسل.

Introduction:

Lumpy skin disease (LSD) is disease of cattle that caused by a capripoxvirus. (LSDV) of cattle, Which is one of three genus of capripoxvirus in family Poxviridae, subfamily chordoxxvirinae(1).

The World Organization for Animal Health (OIE) reported that the latest outbreaks of LSD when occur in Iraq and Turkey, extending concerns that the disease will persist to spread to Asia and Europe. The severity of disease appeared in adult Lactating breeds of cattle in the peak of and breeds of cattle, age and sex are affected (2,3).

The disease is one of the most important economic ailments because of weight gain, decreased milk yield, infertility, and permanent damage to hides. (4).

The clinical manifestation of (LSD) is appear as acute or sub-acute or in apparent depending on the health, breed, immunity status and strain of virus which cause the disease(5).

It is characterized by high rise of temperature, Lymph nodes swell clearly, the nodules on the skin appear as circumscribed, firm and ulcerative (6,7).

The effective method is Vaccination to control of prophylaxis the disease in endemic countries. In countries which LSD one free from

unappearance cases should be confirm by rapid diagnosis is essential so that, such as quarantine, eradication measures, slaughter-out of affected animals and vaccinations can be implemented (8,9).

In affected cattle, LSDV exists in skin nodules, crusts of skin lesions, blood, saliva, nasal discharge, semen and milk (10).

Tentative Diagnosis of LSD is depend upon on characteristic clinical skin lesion signs. However, subacute and inapparent forms require fast and reliable laboratory assay to confirm diagnosis. polymerase chain reaction (PCR) Technique have been developed recently for more accurate and rapid detection of LSDV in suitable specimens (11,12).

The objective of this study was to observation the clinical signs of the cattle infected with LSD and molecular detection of LSDV from clinically suspected skin nodules by using PCR technique as rapid assay to detect the causative outbreak

Of lumpy skin disease.

Materials and Methods

Samples collections: Skin biopsy was collected in 50 ml sterile container from suspected infected cattle with a typical clinical signs of lumpy skin disease. From different field in al-Diwaneya province. then all samples

were transported as soon as possible to laboratory in small cooled box and stored in refrigerator until genomic DNA extraction step.

Histopath procedure : Tissue samples were collected from different animals by using sterile surgical blade and artery forceps by incision (biopsy) 2-4 g and placed in sterile disposable container with formalin 10% for preparation to histopathological sectioning according to (13,14). methods as follows: After that the Tissue samples were fixed by using of formalin (10%) for (48) hrs. then washed in distilled water for three hrs. then the samples were entered to a graded series of increasing ethanol concentration (70%, 80%, 90%, 95% and 100)% for about (2 hrs. for each concentration. The next step the samples were cleared by xylene for three times to 1.5 hr. Then were embedded by liquid paraffin in 56C° for two times. subsequently The samples were Sectioned by the rotary microtome to 5 µm thickness and transported to water bath 50C° and put it on slides which painted with mayor albumin .Blocks samples were deparaffinized by putting them in oven 60 C° for 5 hrs. then put them in xylene for one hour. After that they were dried from xylene, washed with water and put the slides in harris-hematoxylene stain for 15 minutes and washed with flow water. After that Sections were put in acidic alcohol for 5 second, and washed with water until returned of blue color. Then they were put in watery eosin stain for 10 min. and washed with running water Sections were put in xylene for 24 hrs. and mounted the slides with the sticky material and cover slipping with slides covers and left to dry and examined by using the microscope with camera to determine the histological changes , inclusion bodies and skin layer

thickness by optical measure in the microscope lens.

Genomic DNA Extraction: Genomic DNA was extracted from frozen skin lesion samples by using (Genomic DNA Mini Kit, Geneaid. USA). The extraction was done according to company instructions by using Tissue extraction Protocol method with Proteinase K. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored at -20C at refrigerator until used in PCR amplification.

PCR amplification: PCR assay was carried out by using specific primer for P32 antigen gene in Lumpy skin disease virus, the forward primer (CGCGAAATTTTCAGATGTAGTTC CA) and reverse primer (TGAGCCATCCATTTTCCAAC TC)

This primers were designed in this study using (NCBI- GenBank: AF124516.1) and Primer3plus. The primers were provided by (Bioneer company . Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl₂ 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Techne TC-3000. USA) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation

95 °C for 30 s, annealing 58 °C for 30 s, and extension 72 °C for 30 s and then final extension at 72 °C for 5 min. The PCR products (752bp) were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Results and Dissuasion

Clinical and physical investigations:

The first steps of clinical diagnosis were based on clinical examination of infected animals and the result showed

appearance of typical nodular skin lesion (2-5 cm) in diameter which covered all the body and can appear anywhere on the body of animal (figure 1) high rise of temperature (40-41.5°C), drooling of saliva and enlargement of superficial lymphatic vessels and lymph nodes (prefemoral, prescapular lymph node) (figure 2,3) With the Development of the cases the nodules became necrotic, ulcer and eventually a deep scab formed (Fig. 4). and this agreed with (6,15,16).



fig,1: Typical nodular skin lesion cover all the body Of infected animal



fig,2: Enlargement of superficial lymph node (prefemoral lymph node)



fig,3: Enlargement of superficial lymphatic vessels



Fig, 4: Development of the lesion and the nodules became necrotic, ulcer and eventually a deep scab formed

Histopathological results:

Histopathological results showed changes of the affected skin ,epidermis and dermis layers and lesions showed characteristic pathological changes as marked hyperkeratosis (thickening of keratinized layer),also there is ballooning swelling , vacuolation of stratum spinosum layer with downward hyperplasia of stratum basal and infiltration of inflammatory cell in the dermis. Figure (5) were in agreement with (17).Furthermore, there is marked ballooning degeneration of epidermal cell with eosinophilic intracytoplasmic inclusion bodies were also show appear Figure (6,7) this result agreed with (18) Also The result of Histopathological changes of epidermis were similar to other studies which showed swollen cells of the stratum layer and vacuolation and ballooning swelling of spinocytes layer with downward hyperplasia of stratum layer. Figure () they were agreement with (17,18,19),

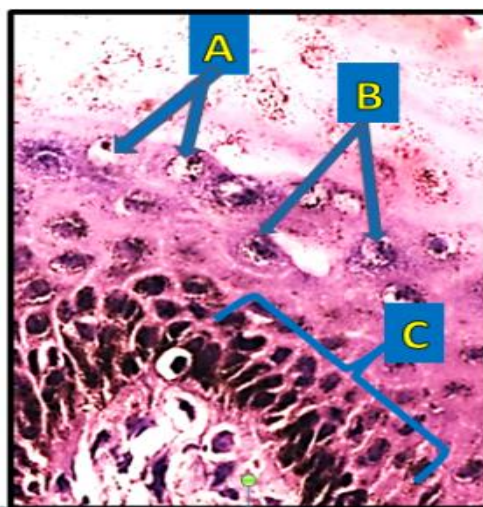


Fig :(5):
 A :Ballooning swelling .
 B: vacuolation of stratum spinosum layer
 C:Downward hyperplasia of stratum basal.

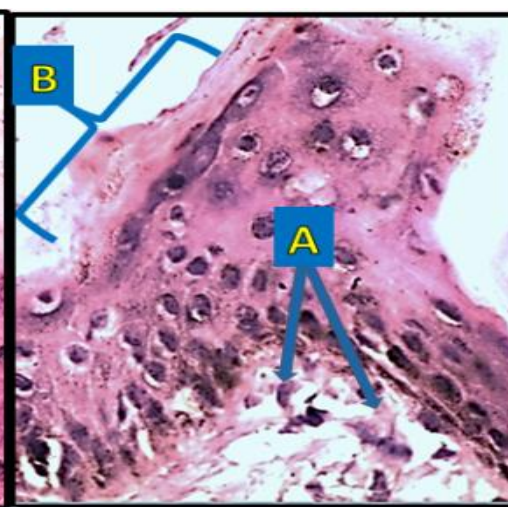


Fig:(6):
 A:Infiltration of inflammatory cell in the dermis.
 B:Marked hyperkeratosis (thickening of keratinized layer).

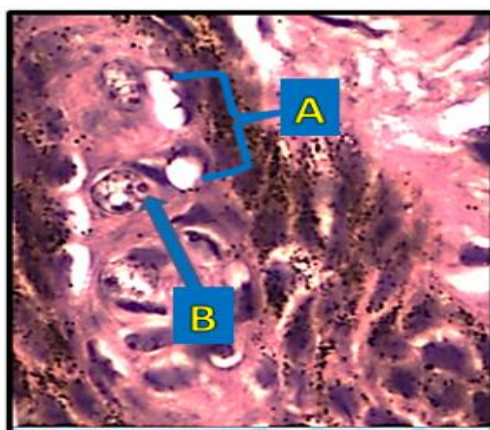
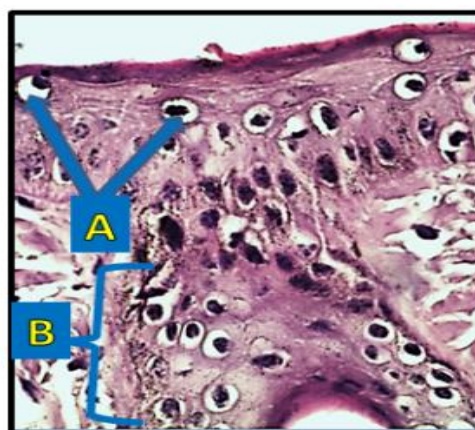


Fig (7):
 A: Ballooning degeneration of epidermal
 B:Esinophilic intracytoplasmic inclusion bodies were also seen



Fig(8) :
 A:Ballooning swelling of spinocytes layer
 B:Downward hyperplasia of stratum layer.

Molecular result : In the current study skin lesion samples were collected showed the presence of capripoxvirus acausative agents to lumpy skin disease in cattle in different age, breed and sex. The result of PCR assay for detection p32 antigen gene of lumpy skin disease virus in ethedimpromide –stained agarose gel by using specific primer for detection p32 gene (720bp), 14 out of 16 were positive result from collected samples figure() this result were agreed with (20)

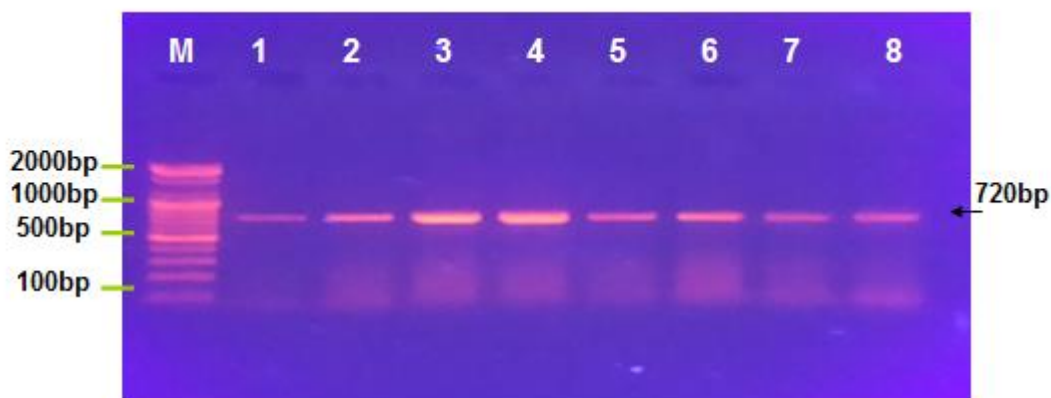


Fig: (9):

Agrose gel electrophoresis image that show the PCR product of P23 antigen gene in lumpy skin diseases at 1% agrose gel.

M: Marker (2000-100bp), Lane (1-8) positive DNA samples from skin lesion samples at 720bp.

In conclusion the lumpy skin disease is wide spread in a AL -Qadissiyya province and the disease have atypical and characteristic clinical ,histopathological feature that used in tentative diagnosis .PCR technique was rapid ,sensitive and specific for detection the (LSD)virus and confirmative diagnosis of disease.

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