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SEROLOGICAL STUDY FOR DETECTION OF NEW EMERGING ECTOPARASITES BORNE DISEASE (*SCHMALLENBERGE VIRUSE*) IN DUHOK PROVINCE - IRAQ

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ABSTRACT

Schmallenberg disease, an ectoparasite borne disease, is one of the most important emerging disease that discovered recently and was first reported in Germany in 2011. The disease affects animal industries through causing many clinical signs ranging from reduce milk production, weight loss to abortion and death of newborn animal. To our knowledge this is considered the first preliminary study on the presence of circulating *Schmallenberg virus* in Iraqi Kurdistan. In this research, only ELISA was used for detection of antibodies and antigens of the virus. Serological study showed that out of 192 animals, 16.1% were tested positive. There was not significantly different between geographical places ($p>0.05$). However, this study showed that the incidence of abortion in sheep due to *Schmallenberge virus* was significantly different between geographical places ($p>0.05$). The overall incidence of abortion in this study was nearly about 47%. The highest incidence was observed in shexan with at 90%.

Key words: *Schmallenberg virus*, Sheep, ELISA, DUHOK

INTRODUCTION

Like other blood feeding Diptera, Culicoides species are vectors of pathogens that can cause a wide varietyof the disease in humans and animals. Biting midges, primarily the species Culicoidessonorensis, are considered a biological vector for transmission of pathogens (like bluetongue virus) in sheep and cattle in the United States of America. Bluetongue is a serious disease of ruminants (Bilk *et al.,* 2012).

Recently, a new virus, which is an RNA virus, belongs to the Simbu-sero-group of the genus Orthobunya virus in the family Orthobunyaviridaeis called *Schmallenberg virus*. The virus is transmitted via biting midges. This new emerging virus causes clinical signs inruminants, such as a drop in milk yield, fever, diarrhea, abortions, and congenital malformations in newborn lambs, kids, and calves (Bilk *et al.,* 2012; Hoffmann *et al.,* 2012; Muskens *et al.,* 2012).

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Hoffmann *et al.* (2012) Reportedclinical signs of unrecognized disease includesharpreductionof milk production, fever, and diarrhea in adult cows in northwestern Germany and Netherlands. Van Den Brom *et al.* (2012) Recorded some other clinical signs among ewes in Netherlands that gave birth of malformed lambs. Those clinical signs have not been reported in Europe previously. However, those ewes did not show any clinical signs during gestation period or at parturition.

Moreover, Helmer *et al.* (2013) described similar cases in goat flocks in spring 2012. In 2012, there were two major outbreaks SBV infection have been reported. In February, the first outbreakreportedin sheep herds and the second outbreak reported in cattle herds in May (Afonso *et al.,* 2014). After these two outbreaks, few months later, the virus had spread over Western Europe with outbreaks in Belgium, France, Germany, Luxemburg, the Netherlands, parts of England and Switzerland. One year later, the disease reached northern Sweden (Conraths *et al.,* 2013). From September, 2011 until April, 2013 more than 8000 laboratory cases had been confirmed with SBV in twenty European member states (Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Hungary, Germany, Ireland, Italy, Latvia,

Luxembourg, the Netherlands, Poland, Slovenia, Spain, Sweden, and the United Kingdom) and two countries in the European Free Trade Association area (Switzerland and Norway) (ANONYMUS, 2013). The number of SBV infected herds may be up to 300 times greater than the number of confirmedreported cases (Garigliany *et al.,* 2012). Furthermore, SBV had been detected in twelve wild species, dog and 19 different species in zoos rather than domestic sheep, goats and cattle (EFSA, 2014).

Antibodies against SBV were found in Turkey (Azkur *et al.,* 2013; TONBAK *et al.,* 2016). Serological detection of SBV in aborted ewes in Duhok province might be explained byanimal importation or the illegal animal trade. The aim of this study here was to analyze samples from ewes and aborted fetuses to investigate the direct presence of SBVin Kurdistan Region /Iraq. Especially when this disease was recorded in Turkey (Azkur *et al.,* 2013; TONBAK *et al.,* 2016; Sukru T. *et al.,* 2013).

MATERIALS AND METHODS

1. Blood Samples

Blood samples were collected from sheep of different age that had history of abortions or fertility problems from different flocks' in Duhok Province, between July and August in Kurdistan region in 2015. A total of 192 serum samples and 192 whole-bloodsamples from 192 sheep were collected intubes without any anticoagulant and in tubes containing EDTA, respectively. For serum, tubes were centrifuged at 3000 rpm for 10 min. All samples were stored in tubesat -20ºC until further analyses.

2. Serological test (ELISA)

The samples were analyzed by the ID Screen *Schmallenberg virus* Competition Multi Species ELISA (ID-vet, France) according to the manufacture instruction (comtet L and Pourquir P 2013). This ELISA is developed for ruminants and other species. Briefly, the ID-vet ELISA testing procedure was as follow, serum, positive (PC) and negative (NC) controls were added to antibodies were present, an antibody-antigen complex, which masks the nucleoprotein epitopes, was formed. After incubation, conjugate that fixes to the free nucleoprotein epitopes was added. After washing steps, substrate solution and finally stop solution were added. The micro-plate was read at 450nm. The optical density (OD) was recorded and the competition percentage (S/N%) where S is the OD of the sample and N is the OD of the negative control, was calculated:

S/N%=OD sample/OD negative control X 100

The results were interpreted as follows: S/N % less than or equal to 40% were considered positive, greater than 50% negative, and between 40-50% as doubtful.

Statistical analysis

Before performing analysis, data were checked for normality and homogeneity of variance using residual plots and Bartlett's test, respectively. The prevalence of *Schmallenberg virus* and the incidence rate of abortion in sheep caused by *Schmallenberg virus* between geographical places were analyzed using logistic regression in Genstat 12th Edition (VSN international Ltd, Hemel Hempsteadf, UK).

RESULTS

Serological method used for detection of new suspected diseases may be recently reached Middle East especially when an outbreak of this disease recorded in Turkey (TONBAK *et al.,* 2016). Table (1 and 2) shows theresults obtained during serological investigation for 192 samples of aborted and non-aborted ewes having history of fertility problems collected from most parts of Duhok province especiallyfrom the areas that are close to Syria and Turkey border, which are the two main illegal entrances of animals. This illegal movement helps to transports some trans-boarding disease.

This study showed that the incidence of abortion in sheep due to *Schmallenberg virus* was significantly different between geographical places (P>0.001; table 1). The overall incidence of abortion in this study was nearly about 47%. There was a wild variation in the reported incidence of abortion between geographical places, with a range of 10-90%. The highest incidence was observed in Shexan with at 90%. It is interesting to note that West of Zakho and south of Sumel have reported the same rate of abortion in sheep (60%) caused by *Schmallenberg virus.*

Table 1: The incidence rate of abortion in sheep caused by *Schmallenberg virus*between different geographical places

Geographical place	No. of aborted	No. of Non-aborted	Total number	% of abortion
West of Zakho	12	8	20	60.0
North of Zakho	16	19	35	45.7
West of Sumel	2	18	20	10.0
South of Sumel	18	12	30	60.0
Duhok	20	10	30	66.7
Shexan	18	2	20	90.0
Amedy	4	33	37	10.8
Total	**90**	**102**	**192**	**46.9**

Also the results demonstrated that the prevalence of *Schmallenberg virus* was not significantly different between geographical places (P>0.05; table 2). The mean prevalence of *Schmallenberg virus* across the whole study was 16.1%. The highest incidence was observed in West and North of Zakho at 30 and 20%, respectively. Thereafter, the prevalence has been lower between 6-16%.

Table 2: The prevalence of *Schmallenberg virus* across different geographical places.

Geographical place	Positive number	Negative number	Total number	%
West of Zakho	6	14	20	30.0
North of Zakho	7	28	35	20.0
West of Sumel	3	17	20	15.0
South of Sumel	5	25	30	16.7
Duhok	2	28	30	6.7
Shexan	2	18	20	10.0
Amedy	6	31	37	16.2
Total	**31**	**161**	**192**	**16.1**

DISCUSSIONS

Schmallenberg virus was first detected in autumn 2011 in dairy cattle in central Europe (Garigliany *et al.*, 2012; Hoffmann *et al.*, 2012; MĂNESCU *et al.*, 2015). It was subsequently found in malformed lambs, kids, and calves in Germany (EFSA, 2014). The speed of SBV spreading across Europe in 2011 and 2012 was impressive, and thus the distribution of SBV to bordering countries was very likely. Currently, in Kurdistan there is no report about the detection of SBV.

Our results showed the competitive ELISA detected about 16.1% positive and its different rates in different area from Duhok Government (Table2). The rate of infection was higher in theareas near to Syrian border, and this rate decreased in far area this depend upon the concentration of imported animals especially illegal imported animal, the illegal movement of animals from Syria was increased after ISIS war.

This study reported the first time the existence of this disease in Kurdistan Region Duhok Governorate. Unfortunately, it is not sure that SBV is present in Kurdistan Region/Iraq since there is a risk for cross reactively with other simbu viruses. By the results in this study, it can only suggest that SBV or an SBV like virus is present in Kurdistan Region/Iraq. To confirm the result of this study, it is recommended to do further study especially in molecular using PCR or RT-PCR for detection of this virus, in addition to this, the sequencing which is important as well to confirm our results and causative agent of this diseasealso recommended.

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دراسة مصلية لكشف اول ظهور لمرض (Schmallenberg) الناقلة عن طريق طفيليات الخارجية في محافظة دهوك – عراق

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يعد مرض شمالنبيرك الناتج عن اصابة الحيوانات بفيروس (Schmallenberg) من الامراض الحديثة التسجيل في العالم وهي من الامراض التي تنتقل بواسطة الطفيليات الخارجية ، وحيث سجلت لاول مرة في المانيا عام ٢٠١١ . وتوثر هذا المرض على انتاج الحيوان وذلك من خلال ظهور عدة علامات مرضية من بينها انخفاض في انتاج الحليب وفقدان الشهية والاجهاض وموت للحيوانات حديثة الولادة. اعتبرت هذه اول دراسة اجريت لبحث عن فيروس schmallengberg (virus) في اقليم كردستان العراق وخاصة محافظة دهوك. وفي هذه الدراسة تم استخدام تقنية ELISA للكشف عن الاجسام المضادة ومستضدات الخاصة لهذا الفيروس . ومن خلال دراسة سيرولوجية (مصلية) سجلت ١٦،١% نسبة الاصابة من مجموع ١٩٢ التي استخدمت في هذه الدراسة. لم تسجل فروقات معنوية مابين المناطق الجغرافية عن وبائية المرض ($p>0.05$) ، ومن جانب اخر سجلت اختلاف معنوي من حيث حدوث الاجهاض في مناطق مختلفة وكانت اعلى نسبة الاجهاض سجلت كانت في منطقة شيخان حيث بلغت ٩٠% .

مفاتيح استلال :- دهوك / اغنام / اليزا / *Schmallenberg virus*