

SERO PREVALENCE OF SCHMALLEMBERG VIRUS ANTIBODIES IN BUFFALO FROM NORTH BASRA GOVERNORATE- IRAQ

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ABSTRACT

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The present study was aimed to record the seroprevalence of Schmallenberg virus (SBV) antibodies in buffalos from North Basra province between 2018 and 2019. A total of 90 serum samples were analysed using a competitive enzyme-linked immunosorbent assay (ELISA). The clinical signs observed in animals suspected to be seropositive were appetite loss (90/90 [100%]), decreased milk production, (82/90 [91.1%]), stillbirth (54/90 [60%]), abortion (35/90 [38.8%]), and malformation (9/90 [10%]). In total, investigation of buffalo's serum samples using competitive ELISA technique revealed a high seroprevalence rate of SBV infection, 43 of 90 (47.7%) tested buffalo's serum samples were positive for SBV antibodies. The seropositive ELISA rates according to clinical signs were 31/82 (38%), 19/54 (35.18%), 11/35 (31.42%), and 5/9 (56%) for decreased milk production, stillbirth, abortion, and malformation, respectively. In conclusion, this is the first report of the seroprevalence of SBV antibodies in buffalo from Iraq. Schmallenberg virus infection severely threatens the health status of local livestock and proper prevention and control measures are required to reduce the morbidity caused by SBV infection.

Key words: Buffalo, SBV, seroprevalence, insect-born disease, competitive ELISA

Introduction

During summer and fall season of 2011, ranchers and veterinarians in Netherlands and northwest Germany reported a newly undiagnosed outbreak having symptoms of diarrhea, increase body temperature with short-term decrease of milk yield (Helmer *et al.*, 2013). During November 2011, the causative agent of these cases was elucidated by the Federal Research Institute of Animal Health in Germany (Friedrich Löffler Institute). Interestingly, dairy cattle blood samples were collected from an infected farm near Schmallenberg at the North-Rhine-Westphalia in Germany, then tested *via* a "meta-genomic" process and "next-generation sequencing", and a new detected virus, tentatively called Schmallenberg virus "SBV" recognized (Hoffmann *et al.*, 2012).

Mostly infected animals were deformed and stillborn, died or euthanized shortly post parturition. Almost entirety of SBV infection, one animal only found to be affected from the multiple births, whereas no deaths or abnormalities were detected in the other animals housed at the self-same bran. The incidence of caesarean section, fetotomy, and delivery assistance were also increased (Van Der Poel *et al.*, 2014). Additionally, increase delivery duration was common, sometimes resulting in placental retention followed dominantly by endo-metritis. Furthermore, deaths of foetuses in large numbers, stillbirth or abortion have been reported (Ganter and Köß, 2012). Infected animals experienced high mortality, decreased milk production, still birth, and abortion, leading to economic losses (Beer *et al.*, 2013). Numerous challenging and debilitating conditions/diseases such as Colibacillosis (Naji *et al.*, 2019), foot-rot (Saleh *et al.*, 2019) and deficiency of vitamin D (Alabada and Saleh, 2020) are strongly affecting the cattle industry including buffalos in Basra, Iraq. However, limited information exists about the characteristics and epidemiology of SBV infection in the Middle East including Iraq.

To our knowledge, no single study has documented the prevalence of SBV in buffalo from Iraq. Therefore, the objective of this study was to investigate the seroprevalence of SBV antibodies in buffalo from North Basra using enzyme-linked immunosorbent assay (ELISA) and study the common clinical signs of SBV infection in buffalo.

Materials and Methods

Study design : This research was done using 90 buffalos aged 3-6 years, according to the rancher reports or estimates given the animal's size or teeth by inspection. A total of 90 buffalo experienced abortion, still birth, decreased milk production, and malformation. The study was conducted between October 2018 and January 2019 in the lactating herds in North Basra Governorate, Iraq. The laboratory analysis was done in the laboratory of "Clinical Pathology", Veterinary Medicine College, Basrah University, Iraq.

Ethical considerations : This study was conducted in accordance with the rules and the guidelines issued by College of Veterinary Medicine, University of Basrah, Iraq. The experiment procedure was approved by the animal care and welfare committee of Veterinary Medicine College, Basrah University.

Questionnaire : Information regarding buffalo's health and symptoms such as abortion, congenital anomalies or decrease in milk production were obtained from ranchers.

Clinical examination : The buffalos that underwent abortion were tested, their vital signs were assessed and recorded following the guidelines of Jackson and Cockcroft (2008).

Serum samples : Blood samples were collected aseptically in plain tubes from the jugular vein of all included animals using a vacutainer system without an anti-coagulant. Then the blood samples were centrifuged at 1500 rpm for 10 minutes, and then serum, plasma, and the buffy-coat were kept by cryo-tubes in deep freezer at - 45°C till the day of the analysis.

ELISA technique : ELISA was performed using anti-Schmallenberg antibodies ELISA kits (Syanova® ELISA/Sweden) by following the manufacturer's instructions. Syanova® ELISA kit is "in-direct ELISA" where the tested antibodies with its conjugate forms the complex. Double sample testing were performed, every single sample was added each sample was added into two different wells; the first was coated with control antigen while the second coated with the Schmallenberg virus antigen. The coated Microplates were read at optical density (OD) 450 nm. Values of the OD were adjusted through subtracts the both wells values of every

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single sample. Positive percentage (PP) values were correlated with the positive control value.

$$PP = \frac{ODCorr(\text{sample or NC})}{ODCorr(\text{PC})} \times 100$$

PP < 10 was considered as negative and PP > 10 as positive.

Statistical Analysis : The data of the current study was analysed using "Microsoft Excel" software and/or "Chi Square Test" for evaluating the seropositivity status and the percentages of the common clinical signs of SBV infection in lactating buffalos bred in North Basra, Iraq

Results and Discussion

The ELISA test analysis of the current study showed higher rate of the seroprevalence of SBV infection in buffalos, the seropositive percentage of antibodies against SBV were 43/90 (47.7%) (Table 1). The clinical signs were as follows: loss of appetite (90/90 [100%]), decreased milk production, (82/90 [91.1%]), still birth (54/90 [60%]), abortion (35/90 [38.8%]), and malformation (9/90 [10%]) (Figure 1). In total, the results of ELISA show the seropositive sample were 43/100 (47.7%) for (SBV) antibodies (Figure 2). Of the buffalo positive for SBV, 31 (34.44%), 19 (21.11%), 11 (12.2%), and 5 (5.55%) had decreased milk production, still birth, abortion, and malformation, respectively (Figure 3). The seropositivity rates according to clinical signs were 31/82 (38%), 19/54 (35.18%), 11/35 (31.42%), and 5/9 (56%) for decreased milk production, still birth, abortion, and malformation, respectively (Figure 4).

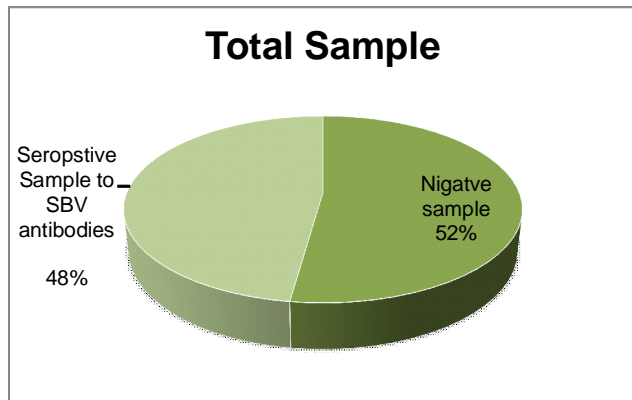


Fig. 1: Total per cent to seropositive sample for SBV antibodies

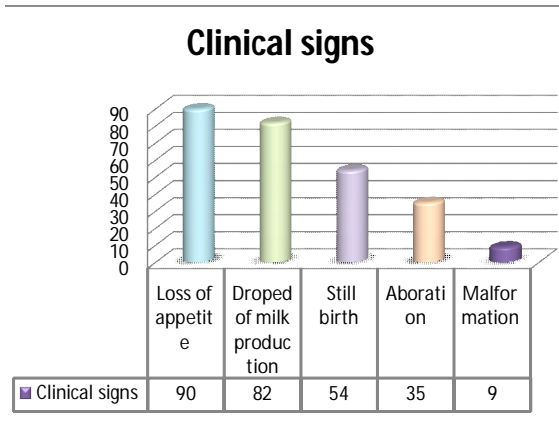


Fig. 2: Clinical signs in all buffalo

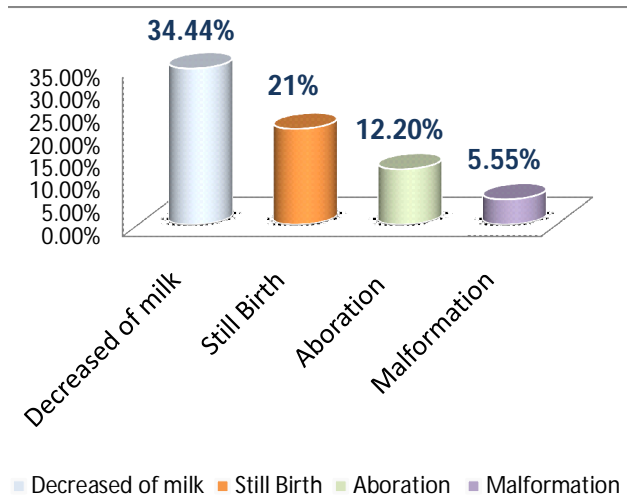


Fig. 3: Clinical signs in seropositive buffalo

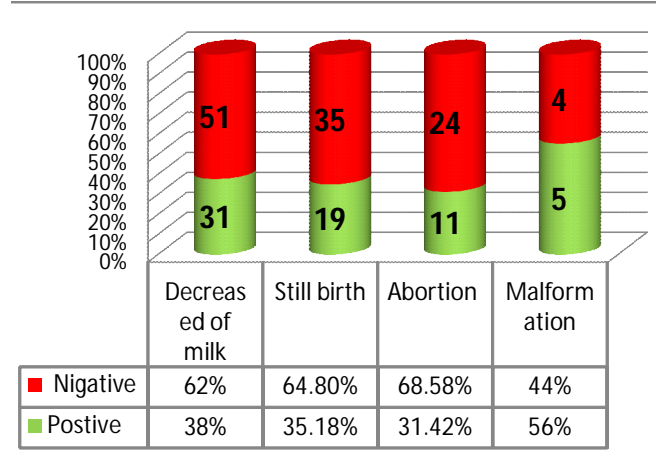


Fig. 4: Seropositivity rates according to clinical signs

To the best of our knowledge, this is the first report on SBV antibodies in buffalo from Basra Governorate, Iraq. The results of our study show that there is a high correlation between the seroprevalence rate of buffalo flocks in which the presence of SBV was confirmed by competitive ELISA and the appearance of the illness such as abortion, still birth, malformation, decrease milk production and inappetence.

Despite the number of the animals in the current study was limited, our findings demonstrate that the SBV seroprevalence was extremely high in buffalo with sharp decrease of milk production suggesting that the drop in milk yield is the most dominant sign of SBV infection in buffalos. However, unspecific clinical signs including fever, diarrhea, abortion, and decrease milk production were recorded in adult SBV affected cattle (Gibbens, 2012; Hoffmann *et al.*, 2012; Afonso *et al.*, 2014). The current screening study was not able to give very precise estimates for the buffalo population, but give an indication that the SBV infection in this species might be higher or lower. This would mean that a considerable portion of buffalo population as well as cows, sheep, and goat populations remain susceptible and might be maintain the infection in Basra or other parts in Iraq. Therefore, it seems probable that the SBV infection

in this region with high seroprevalence rate has spread much faster to animals located in areas are still free from SBV.

As the possible role of wind transmission of SBV is existed and the virus can be transmitted *via* wet wind and/or infected insects among hundreds kilometres (Sedda and Rogers, 2013; McGrath *et al.*, 2018), it seems reasonable to suggest that the SBV infection was introduced to Iraq *via* wind-born spread from the neighbour endemic countries such as Turkey. However, the seroprevalence rate (48%) for SBV antibodies in local Iraqi buffalos was higher than buffalos in other countries such as Turkey and china (Tonbk *et al.*, 2016). The differences between the results of this study and those of previous studies may be due to differences in location, species of buffalo, and sampling method (random sampling was used in the Turkish study). Furthermore, artificial insemination could have a suspicious rule of SBV transmission (Schulz *et al.*, 2014; Sibhat *et al.*, 2018). The SBV contaminated semen that imported from European countries (such as Germany, Netherlands, and France) may easily infect the local Iraqi cows, which eventually transmit the infection to buffalos *via* mosquito bites. Nevertheless, Iraq has no control programmes for the high population of mosquitos in its marshes; this may have led to the higher seroprivalence rate. Transboundary movement of animals is not excluded as a significant source of SBV infection.

Based on the reports of the European Food Safety Authority, the SBV infection has been given the status of a notifiable animal disease in some European countries such as Germany (Helmer *et al.*, 2013) and the Netherlands (Veldhuis *et al.*, 2013), while it is not in Belgium (Méroc *et al.*, 2014) and other countries. Considerable in Germany alone, 1048 herds of cattle, 880 herds of sheep, and 48 herds of goats have been reported to be infected with SBV, with high estimates of un-reported cases (Helmer *et al.*, 2013). In the mean time and through Friedrich-Loeffler-Institute (FLI), numerous countries (Finland, Norway, Northern Ireland, Sweden, Ireland, Spain, UK, Italy, Switzerland, Denmark, Poland, Luxembourg, Belgium, Austria and France) bordering Netherlands and Germany have reported confirmed cases of Schmallenberg virus infection (Rasmussen *et al.*, 2012).

Related studies done by De Regge *et al.* (2013) Méroc *et al.* (2014) Wernike *et al.* (2014) indicated that SBV infection is molecularly detected from malformed aborted, and stillborn samples obtained from lambs and goats more often than from calves. This could be due to the long pregnancy period of cows than in sheep and goats. However in cattle, the offspring is no longer infected at the time of delivery.

In conclusion, the results of the current study provide evidence for the first time that a part of buffalo population in Basra region had high SBV seroprevalence rate that causes significant economic losses. Thus, almost every buffalo, cow, sheep and goat have been at the same point in contact with SBV infection and the animals located in free SBV areas could in turn to become infected. Our findings should be taken into consideration as the first line of SBV investigation strategy reaching to control and eliminate this disease in Iraq. Further wide range screening tests combined with surveillance actions are crucially needed for monitoring the SBV and the other insect-borne infections.

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Conflict of interest

The authors declare that they do not have any financial or personal relationships that might have affected them inappropriately in writing this article.

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