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Research Article

Sero-Prevalence and Risk Factors for Sheeppox in Kordofan States in Sudan

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ABSTRACT

Background: Sheeppox and goatpox are viral diseases of sheep and goats causing high morbidity and mortality leading to large economic losses for producers. The viruses are transmitted primarily through direct contact between infected animals. Understanding the sero-prevalence, risk factors and producers knowledge of the disease is critical for implementation of control strategies

Methods: A cross-sectional survey was performed in the Kordofan region, from March to September 2011 using a Virus Neutralization Test (VNT) and ELISA. The serology data was used to identify potential risk factors associated with sheep pox outbreaks. In addition, a questionnaire explored producer's knowledge about the disease in the Sudan.

Results: The estimated overall sero-prevalence of sheeppox in the Kordofan region was 73.4% determined by virus neutralization and was prevalent in both South and North Kordofan states at 85% and 64% respectively. However, the seroprevalence determined using ELISA of sheeppox in South and North Kordofan states was 33% and 15% respectively. The risk factors identified were the breed, age, sex, species, movement patterns, herd size and geographic region. The questionnaire revealed that both nomadic and permanent farmers were generally aware of sheeppox as a disease, but most did not have a complete understanding of the disease. Greater than half of producers experienced the disease in the past 2 years and did not have their sheep vaccinated.

Conclusion: This study illustrates the disease burden of sheeppox in Sudan and demonstrates that for sero-surveillance, VNT is a more sensitive method compared to ELISA for detecting previously infected animals. Further education of producers of the disease and important of vaccination is required to control the disease.

Keywords: Capripoxvirus; Sheeppox; Sero-prevalence; ELISA; Surveillance; Risk factors; Sudan

INTRODUCTION

The genus capripoxvirus of the Poxviridae family consisits of three members, sheeppox virus, goatpox virus and lumpy skin disease virus of cattle. Sheep and goat pox are viral diseases of sheep and goats causing high morbidity and mortality leading to large economic losses for producers. Sheeppox and goat pox are generally host specific, however there are some isolates which can cause disease in both sheep and goats [1]. The clinical signs of sheep and goat pox are fever, nasal discharge, external skin lesions as well as internal lesions in lungs and gastrointestinal tract and the disease is spread through a combination of aerosol transmission and contact with infected animals. Sheep and goat pox are widespread in most of Africa, the Middle East, Turkey and Asia including India and China [2,3] and have increased their geographic range with recent outbreaks in Greece, Vietnam [1], Mongolia [4] and Russia [5]. Capripoxviruses are highly conserved at the genetic level with 97%-99.9% homology between different isolates [6]. The genetic determinants responsible for the host specificity are not fully understood [7]. There are no serotypes for capripoxvirus, and therefore, serology cannot be used to identify the specific capripoxvirus member. Control of capripoxvirus can be achieved using live attenuated vaccines [8].

Serology can be used to identify animals previously infected with capripoxviruses. The Virus Neutralization Test (VNT) is the currently used gold standard test to detect antibodies specific to capripoxvirus in animals to determine if they have been infected [9]. Unfortunately the virus neutralization test is labour intensive and slow to generate results. There are several capripoxvirus ELISA

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that have been described including an ELISA based on purified inactivated capripoxvirus [1] as well an indirect ELISA based on two viral core antigens [10]. A commercial indirect Capripox Double Ag ELISA is available from ID-VET (ID-VET), which is a suitable ELISA for detecting anti-capripoxvirus antibodies [11].

The sheep industry in Sudan is a major contributor to the economy and is one industry which allows citizens to generate income and alleviate poverty. Sheeppox in Sudan is responsible for dramatic economic losses, particularly for animals exports. In Sudan, sheeppox identified to be endemic since 1944 [12]. In Sudan, goat pox caused clinical disease in both sheep and goats [13,14]. The current epidemiological situation has changed in Sudan where sheeppox virus is present and this virus causes disease only in sheep [15]. The aim of this paper was to understand the epidemiological risk factors as well as the sero-prevalence of sheeppox in Kordofan regions as well as producer's knowledge about the disease. This understanding will be used to educate farmer and to promote vaccination allowing for improved animal health and economic development.

METHODS

Study area

The study was conducted in North, Western and South Kordofan States. Kordofan covers an area of 376,145 km² (146,932 miles²), with an estimated population of 3.6 million people in the 2000 Census (Figure 1). It is largely an undulating plain, with the Nuba Mountains in the southeast quarter. During the rainy season, the area is fertile, but in the dry season it is virtually desert [16].

Study population

The study focused on sheep herds as reference target population. The total population of sheep in Kordofan region is 2,759,124 heads, 30% are Hamari sheep and 70% other breeds of local Sudanese sheep [17].

Sample size determination

A cross-sectional study design was carried out from March 2011 to September 2011 to determine the sero-prevalence of sheep pox in Sudanese sheep flocks in Kordofan. A multistage sampling technique was carried out. Starting from the region and the 2 states, localities were purposively selected, then flocks within a locality were purposively selected and individual animals within the flocks were sampled.

The sample size was calculated for an expected prevalence of 63.5% in a locality, according to a previous study by Eshafi and Ali [18], using a 5% desired absolute precision and a 95% confidence level. Farmer's permission to use their animals was clarified by communicating to local leaders before approaching farmers. A total of 1500 animals were sampled in the 6 localities in North and South Kordofan States, due to lack of stock of ELISA test at time of the study only 850 samples were evaluated by ELISA. While, 260 samples were shipped to National Centre for Foreign Animal Disease, Winnipeg, MB, Canada, and assessed using virus neutralization. Risk factors were identified as individual animal risk factors including: sex, age group, weight, species and breed; other potential risk factors were location, herd size, movement pattern, mixed herd, agri-ecologic zone, temperature and relative humidity.

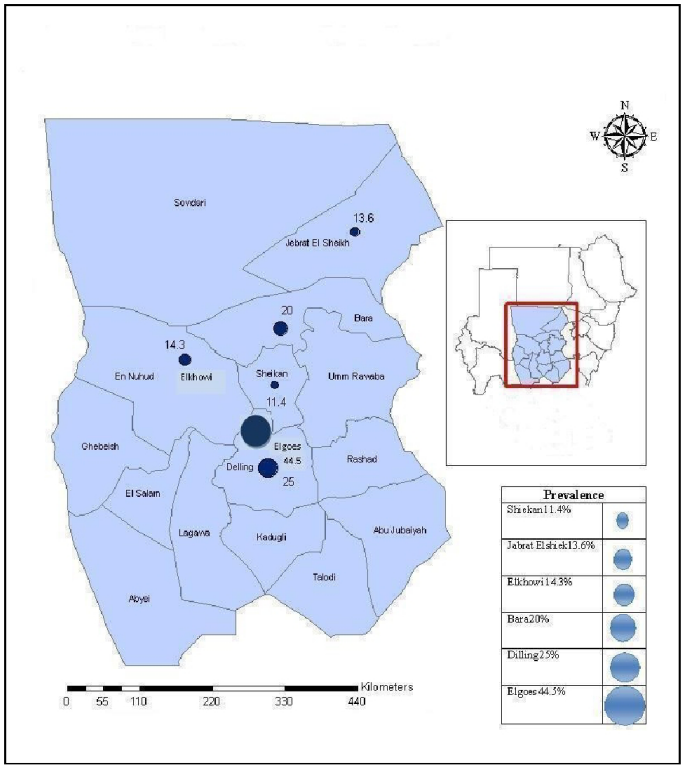

Figure 1: Map of North and South Kordofan States where the study was conducted.

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Serum sample collection

Only non-vaccinated sheep flocks were used to collect blood. Blood samples were collected from sheep using plain 10 ml vacutainers and 19 gauge needles (Bectin Dickinson, UK). The collected blood was allowed to clot for up to 24 hours in the shade. Two aliquots of sera were transferred into cryovials labeled and were kept in a -20°C freezer and transferred to the laboratory in cooled containers with ice bags. Serological diagnosis was carried out on the collected specimens at the Veterinary Research Institute (VRI), Department of Virology, and Department of Viral Vaccine Production, Sudan and the National Center for Foreign Animal Disease (NCFAD) in Canada.

Virus neutralization test

Sheeppox virus specific antibodies were determined using a Virus Neutralization Test (VNT). The assay was performed using negative and positive control sera as well as the test sera which were heat inactivated at 56°C for 30 minutes prior to performing the assay. Sera were diluted starting at 1:10 in DMEM and then serially diluted by a factor of 2. After dilution, the 125 µl of diluted sera was added to a 96 well microtitre plates containing 125 µl of 100 TCID50 per 100 µl of Kenya sheep pox virus. The plates were incubated at 37°C for 1 hr. After this period media was removed from confluent OA3.Ts cells where obtained at Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, Winnipeg, MB, Canada, which had been seeded 24 hr previously on 96 well microtitre plates, 200 µl of the virus/sera mixture was added and incubation was continued for 6 days. Development of CPE was then scored using an inverted microscope. Neutralization was considered positive if greater than a 50% reduction in CPE was observed at a dilution of 1:10 or greater [19].

Capripox double antigen ELISA

Ovine samples were examined to detect antibodies against sheeppox using a capripox double antigen ELISA according to the manufacturer instructions (ID-VET, France).

Producer questionnaire

Data from North and South Kordofan States were collected by questionnaires filled out by farmers. Other information and secondary data for the questionnaire was taken from General Planning and Animal Resources Economics Administration, Federal Ministry of Animal Resources and Fisheries of the Sudan. The method of the questionnaire were used according to guideline and regulation of Federal Ministry of Animal Resources and Fisheries, Sudan and consent of respondent to participate in the current study. All experiments were carried out according to regulation and guidance of Central Veterinary Research Laboratory (CVRL), Canadian Food Inspection Agency (CFIA), and Australian Animal Health Laboratory (AAHL). Also, information was obtained from respondents or and/informants under 18 with their parents or and/guardian.

Statistical analysis

Collected data were organized and managed in a Microsoft Excel spreadsheet for analysis by the Statistical Package for Social Sciences (SPSS) at Central Veterinary Research Laboratory, Sudan and by Prof. Moses Kuyle at Addis Ababa university, faculty of Veterinary Medicine at Debre Zeit. The results of statistical analysis was extrapolated for the sample size difference. The risk factors associated with sheeppox seropositivity, were analyzed using descriptive statistics and univariate analysis chi-square test.

RESULTS

The overall sero-prevalence determined using a Virus Neutralization Test (VNT) in North and South Kordofan States was 73.4% with a sero-prevalence in the North of 63.6% and in the South of 85% (Table 1). Different regions had different levels of sero-prevalence determined by VNT ranging from 60% in Elkhowi to 98.3% in Dilling. The seroprevalence determined using ELISA of sheeppox in South and North Kordofan states was 33% and 15% respectively (Table 2). Different regions had different levels of sero-prevalence determined by ELISA ranging from 14% in Jabrat Elshiek to 45% in Elgoes.

Individual animal risk factors

Individual animal risk factors such as sex, age and weight were not associated with the sero-prevalence of sheeppox in the Sudan consistently between the different regions, indicating that these factors do not influence sheeppox infection. However the breed of sheep was significantly associated as a risk factor with the Gharaj breed showing the highest level of seroprevalence of 84.2% (Table 3). The age and sex were not significantly associated with seropositivity by ELISA ($P>0.05$), whereas, species and location were significantly associated with its seropositivity $P<0.05$ in Kordofan states. Also, comparing to VNT, study was investigated that, state, locality, breed, herd type, herd size, movement pattern, agri-ecological zone and insect bites were significantly associated with seropositivity of sheeppox ($P<0.05$), while temperature and relative humidity were not associated with seropositivity of sheeppox ($P>0.05$) using VNT.

Table 1: Seroprevalence in the North and South Kordofan States evaluated by virus neutralization.

State	Locality	Total number of samples	Number of positive	Prevalence (%)
N. Kordofan	Jabrat Elshiek	140	89	63.6
		70	47	67.1
	Elkhowi	70	42	60
S. Kordofan	Dilling	120	102	85
		60	59	98.3
	Elgoes	60	43	71.6
	Overall prevalence	260	191	73.4

Table 2: Seroprevalence in the North and South Kordofan state by ELISA.

State	Locality	Total number of samples	Number of positive	Prevalence (%)
N. Kordofan	Jabrat Elshiek	350	54	15.4
		250	34	13.7
	Elkhowi	100	20	20.2
S. Kordofan	Dilling	500	164	32.8
		300	75	25.2
	Elgoes	200	89	44.5
	Overall prevalence	850	218	25.6

Table 3: Husbandry and ecological risk factors and their association with sero-prevalence of sheeppox.

Risk factor		VNT		Chi square	df	P-value
		Negative	Positive			
State	N. Kordofan	69(49.3%)	71(50.7%)	32.294a	1	0.001
	S. Kordofan	19(15.8%)	101(84.2%)			
	Total	88(33.8%)	172(66.2%)			
Locality	Jabart Elshiek	29(41.4%)	41(58.6%)	46.910a	3	0.001
	Elkhowi	40(57.1%)	30(42.9%)			
	Dilling	1(1.7%)	59(98.3%)			
	Elgoes	18(30%)	42(70%)			
	Total	88(33.8%)	172(66.2%)			
Breed	Khabashi	27(42.9%)	36(57.1%)	38.211a	2	0.001
	Hamari	38(59.4%)	26(40.6%)			
	Gharaj	19(15.8%)	101(84.2%)			
	Total	84(34%)	163(66%)			
Herd type	Pure sheep	81(67.3%)	120(59.7%)	19.029a	1	0.001
	Mixed	3(6.5%)	43(93.5%)			
	total	84(34%)	163(66%)			
Herd size	51-100	27(20.5%)	105(79.5%)	29.710a	2	0.001
	101-150	20(69%)	9(31%)			
	>150	37(43%)	49(57%)			
	Total	84(34%)	163(66%)			
Movement pattern	Nomadism	19(15.8%)	101(84.2%)	34.351a	1	0.001
	Sedentary	65(51.2%)	62(48.8%)			
	Total	84(34%)	163(66%)			
Agri-ecological zone	Arid	27(42.9%)	36(57.1%)	38.211a	2	0.001
	Semi arid	38(59.4%)	26(40.6%)			
	Sub arid	19(15.8%)	101(84.2%)			
	Total	84(34%)	163(66%)			
Insect bites	Yes	39(52%)	36(48%)	15.847a	2	0.001
	No	10(30.3%)	23(69.7%)			
	No answer	35(25.2%)	104(74.8)			
	Total	84(34%)	163(66%)			
Temp°C	High	27(42.9%)	36(57.1%)	2.951a	1	0.086
	Moderate	57(31%)	127(69%)			
	Total	84(34%)	163(66%)			
Relative humidity	Moderate	57(31%)	127(69%)	2.951a	1	0.086
	Low	27(42.9%)	36(57.1%)			
	Total	84(34%)	163(66%)			

Note: aSignificant by univariate analysis using Chi square

Other risk factors

Other risk factors that were identified were herd type, herd size, movement patterns, agri-ecological zone and the absence of insect bites. Mixed herds of sheep and goats compared to herds consisting of only sheep had higher levels of sero-prevalence. A small herd size ranging from 1-50 animals had the highest sero-prevalence rate compared to larger size herds. Nomadic herds had a higher levels of sero-prevalence compared to sedentary herds. The agri-ecological zone was identified as a risk factor with sub arid zones having the highest sero-prevalence rate.

Questionnaire survey about animal production practices

The questionnaire survey revealed that the production system of sheep herders in North and South Kordofan was 77% sedentary and 23% nomadic herds. The informants were asked for their consents to voluntary giving their responds in this study. Most of the sheep herders interviewed were found to be aware general clinical disease of sheeppox, with 68% of them were aware of skin lesion, and 13% aware of respiratory signs, while only 9% were aware of digestive distress. In addition, a high percentage of producers surveyed, recently experienced sheeppox with 59% of producers reported having the disease in the last 2 years, 82% of producers reported having the disease in the last 5 years and 100% of producers reported having the disease in the last 10 years. For vaccination, only 9% of herders had used vaccination against sheeppox in their flocks. For treatment of sheeppox, quarantine is rarely applied with only 16% of the respondents using quarantine as control measure against sheeppox consisting of a mix of strategies including isolation of infected animals, treatment and restricted movement. Traditional treatment for sheeppox using an acacia species emulsion was practices by 12% of respondents who claimed that it was as efficient as modern veterinary practices like vaccination. In regard to the age which sheep are most susceptible to sheeppox, 82% were of the opinion that sheeppox affected all sheep regardless of age with 14% indicating that young sheep were most susceptible and 5% of the respondents thought that adult sheep were most susceptible.

DISCUSSION

The overall sero-prevalence of sheeppox in the Kordofan region was 73% determined using the virus neutralization test. However, sero-prevalence of sheeppox in the Kordofan region was 26% determined using ELISA. Comparing the sero-prevalence rates to the results from the producers survey, indicate that the VNT results are in agreement with the percentage of producers experiencing the disease. The discrepancy of the results between the VNT and ELISA can be explained by the different antigens which are targeted by each test. The VNT measures antibodies specific to capripoxvirus surface antigens whereas the ELISA measures antibodies specific to 2 core antigens. The cut off used for determining positive samples by VNT was a sera dilution of 1:10 which measures low levels of capripoxvirus neutralizing antibodies which is much more sensitive compared to the a neutralization index of 1.5 [10]. The duration that capripoxvirus core specific antibodies last is currently not known and it is possible that these antibodies are not as long lived as antibodies specific to capripoxvirus surface antigens. The ELISA is suitable for detecting antibodies generated from recent infections and the VNT is able to detect antibodies from recent and older infections. It is interesting that both the VNT and ELISA demonstrated that there were higher levels of seroprevalence in South Kordofan compared to North Kordofan.

In Sudan, control measures for sheep and goat pox start with reporting of new cases to the veterinary authority, followed by a disease investigation and reporting. Control of sheep and goat pox is done using vaccination which is economical and effective when used [20].

The current study demonstrates that sheeppox is prevalent in both the South Kordofan state and in the North Kordofan State. In addition, a number of risk factors such as breed, geographic region, herd size and movement pattern were found to be significantly associated with sheeppox sero-prevalence in the Kordofan region. It is not surprising that different breeds of sheep may be more susceptible to sheeppox. A small flock size between 50-100 head was identified as a risk factor. The smaller the flock size was the greater risk sheeppox could spread in a close flock space, agreeing with previous studies [13,21]. It was observed in Kordofan that nomadic herds had a greater sero-prevalence of sheeppox sero-prevalence compared to fixed farms. In a previous study the risk of sheeppox positivity was almost four times higher in transhuman flocks compared to sedentary flocks [22]. The increased movement of animals likely contributes to the mixing of flocks increasing the spread of sheeppox. The presence of insect bites was found to decrease the sero-prevalence of sheeppox, although the role of insect vectors is currently unclear for the transmission of sheep and goat pox. Since arthropod vectors are not required for transmission of sheep and goat pox, it is likely that the presence of insect vectors is not a major risk factor for the spread of sheeppox in study area. Temperature and relative humidity were not significantly associated with the sero-positivity of sheeppox. This is in contrast to lumpy skin disease virus where the disease occurs following rainfall in the wet season when arthropod vectors are present to transmit the disease.

The questionnaire survey revealed that 68% of the respondents were familiar with the skin lesions of sheeppox, in agreement with a previous study [23]. Forty percent of the respondents used antibiotic treatment as first choice, which might help preventing secondary infections. There was a very low level of vaccination used by farmers in the Kordofan region, with only 9% of the respondents in this study having previously used vaccination against sheeppox in their flocks. The low level of vaccine use is likely due to farmer's opinion that the cost of treatment is cheaper than that of vaccination. However, since veterinary services are public sector, all the services provided by the government including vaccination and treatment are subsidized. Lately, privatization of veterinary services has surged which influence the farmer's opinion not to use vaccines as they cost money. This is in agreement with a study performed to analyze the socioeconomics impact of capripoxvirus in Nigeria which evaluate the economic loss due to seroprevalence of the disease and the impact of livelihood on the owner and Gross Domestic Product (GDP) [24]. A sheeppox vaccine is provided at a reasonable price to the farmers because the vaccine was developed and manufactured in Sudan. These results indicate that it of outmost importance to educate producers about sheeppox and the value of using vaccination to prevent the disease and decrease production losses. Improved multivalent vaccines based on capripoxvirus are currently being developed to protect against peste des petits ruminants and Rift Valley fever virus [9] to provide a cost effective disease solution for these farmers.

CONCLUSION

The current study revealed that sheeppox is prevalent in both in South Kordofan and North Kordofan. This study illustrates the disease burden of sheeppox in Sudan and demonstrates that for sero-surveillance, VNT is a more sensitive method compared to ELISA for detecting previously infected animals. Further education of producers of the disease and important of vaccination is required to control the disease.

DECLARATIONS

This research work is done to fulfil a partial requirement of degree of master degree.

Ethical approval and consent to participate

This study was carried out according to regulation of Freie Universität-Berlin. It had been done in Central Veterinary Research Laboratory Central Veterinary Research Laboratory (CVRL), Animal Resources Research Corporation with collaboration with Canadian Food Inspection Agency (CFIA) and Australian Animal Health Laboratory (AAHL). Also permission to used sera sample from sheep was obtained from Ministry of Animal Resources and University of Sudan for Science and Technology, Faculty of Veterinary Medicine, addressing local leaders and animal owners. Federal Ministry of Animal Resources, Fisheries and Rangelands had legalized and licensed using of sera samples collected to carry out this study on live vertebrate and sheep in the study area for purpose to partial fulfillment of master degree. All methods were used according to relevant guidance and regulation of Addis Ababa University and Freie Universität-Berlin and Sudan University of Science and Technology. All experiments were carried out according to regulation and guidance of CVRL, CFIA, and AAHL. Also, this study was comply with ARRIVE guideline and regulation.

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Authors' contributions

The study was conceived, designed and critically revised by all authors. Laboratory data generation and data analysis was performed by MM, TM and SB. The questionnaire and statistical analysis was performed by MM. MB proposed the study, GA helped in the Methodology, TA assisted in VNT test, FA and AA carried out VNT test, AAM participated in study design, M K helped in statistical analysis, YA helped in ELISA test, KH suggested and revised the study. The drafting of the manuscript was performed by MEA and SB. All authors have read and approved the final manuscript. Map of study area was prepared by Directorate of Animal epidemiological disease and prevention, Federal Ministry of Animal Resources, Fisheries and Rangelands.

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