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Sero-Prevalence and Risk factors for Sheep Pox and Lumpy Skin Disease and Their Comparison to Capri Pox Double Antigen Multispecies ELISA in Khartoum and Kordofan States in Sudan

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Abstract

A cross-sectional survey was performed in the Kordofan region, from March to September 2011 was compared to Capripox Double Ag ELISA for multispecies. The estimated overall sero-Prevalence of sheep pox in Kordofan region was 73.4% determined by virus neutralization and was prevalent in both South and North Kordofan states at 85% and 63.6% respectively. However, Sero-Prevalence for lumpy skin disease was 5% and 62% for sheep pox by using Capripox Double Ag ELISA. The serological information was used to identify potential risk factors associated with sheep pox outbreaks. The risk factors identified were the breed, age, sex, species, movement patterns, herd size and geographic region. In addition, a questionnaire explored producer's knowledge about the disease in the Sudan. The results of the questionnaire were that both nomadic as well as fixed farmers were generally aware of sheep pox as a disease, but most did not have full knowledge about the disease. Greater than half of producers experienced the disease in the past 2 years and did not have their sheep vaccinated.

Keywords: Seropositivity; ELISA; Poxvirida; Vaccine; Virus neutralization

Introduction

Sheep Pox, Goat Pox and Lumpy Skin Disease are viral diseases of sheep and goats and cattle characterized by fever, generalized papules, vesicles, internal lesions (particularly in the lungs), and death. The genus Capripoxvirus of the Poxviridae family comprises of three members, Sheep Pox Virus (SPPV), Goat Pox Virus (GTPV) and Lumpy Skin Disease Virus (LSDV) of cattle. Sheep pox, goat pox and lumpy skin disease are widespread in Africa, Middle East, Turkey and Asia including some former Soviet Republics. Sheep and goat pox are increasing their geographic range with recent outbreaks in Greece, Vietnam, Mongolia and Russia [1].

Capripoxviruses are genetically related, varying between 97%-100% between different capripoxviruses at the genetic level. Capripoxviruses are highly conserved at the genetic level, there are no serotypes for capripoxvirus, and therefore, serology cannot determine the identity of the virus. Virus neutralization is the currently used gold standard test to detect antibodies specific to capripoxvirus in animals to determine if they have been infected. Unfortunately the virus neutralization test is not a user friendly test since it is labour intensive and takes one week to generate results. The lack of a validated ELISA has hampered serological studies on capripoxvirus and its Sero-prevelance in endemic countries. There are several capripoxvirus ELISA that have been described including an ELISA based on purified capripoxvirus as well as different capripoxvirus antigens. Unfortunately the ELISA based on purified capripoxvirus is not ideal since the purification of the virus for antigen is labour intensive requiring ultracentrifugation and has difficulties with quality control of the antigen. An ELISA based on recombinant viral core antigens works well in detecting antibodies shortly after infection but cannot be used for serological studies as the antibodies generated to these antigens are transient. A next generation capripoxvirus ELISA based on a mammalian expressed capripoxvirus membrane protein is currently in development to improve diagnostics. The sheep industry in the Sudan is a major contributor to the economy and has the ability to generate income and alleviate poverty for its citizens. In the Sudan, the first scientific investigation on SPPV was evaluated by who affirmed that the disease was endemic in the country as well as the virus was host-specific. In the Sudan both sheep and goats in a mixed farm had disease caused by sheep and goat pox could infect goats, and GTPV could infect both sheep and goats. Sheep pox in the Sudan is responsible for dramatic economic losses, particularly for animals exports reported alteration of some epidemiological patterns and virus heterogeneity recently observed in sheep pox outbreaks in the Sudan. The aim of this paper was to understand the epidemiological risk factors as well as the Sero-Prevalence of SPPV in Khartoum and Kordofan region and producers knowledge about the disease. This will be used to promote education of farmers and promote vaccination to improve animal health and economic development [2].

Materials and Methods

Study area

The study was conducted in Khartoum state where it covers an area of 22,142 km2. It lies between longitudes 31.5 to 34ºE and latitudes 15 to 16ºN. There are the most populous state with (5,274,321).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. The region's chief town is Al-Ubayyid. Traditionally, the area is known for production of gum Arabic and other crops including groundnuts, cotton, and millet were cultivated [3].

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

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 [4].

Sample size determination

A cross-sectional study design was carried out from March 2011 to September 2011 to determine the Sero-prevalence of SPP 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 conveniently sampled. Bovine sera were conviently collected from farms in Khartoum state [5].

The sample size was calculated for an expected prevalence of 63.5% in a locality, according to a previous study by using a 5% desired absolute precision and a 95% confidence level. Before, farmer's permission to use their animals was clarified by communicating local leaders to facilitate approach to farmers. A total of 2,142 serum samples were to be collected. Accordingly, 1500 animals were sampled in the 6 localities in NKS and SKS and 800 animals were evaluated by ELISA. While, 400 samples were shipped to National Centre for Foreign Animal Disease, Winnipeg, MB, Canada, 260 animals were sampled for evaluation by Virus Neutralization test, 40 Bovine sera were examined for lumpy skin disease and 52 ovine sera were retested for sheep pox by using Capripox Double Ag ELISA. Risk factors were identified as individual animal risk factors which are: 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 [6].

Serum sample collection and testing

Only non-vaccinated sheep flocks were used to collect blood, however, Bovine were reported with vaccination of Lumpy skin disease from kordofan and khartoum respectively. Blood samples were collected from sheep using plain 10 ml vacutainers (BD-Plymouth.Pl6 7BP.UK) and sterile needles (19 gauge; for each animal) to minimize pain or discomfort. 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, Department of Viral Vaccine Production, Sudan and National Center for Animal Disease (NCFAD) in Canada [7].

Virus neutralization assay

Sheep pox virus specific antibodies were tested by using Virus Neutralization test. 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 h. After this period media was removed from confluent OA3.Ts cells, which had been seeded 24 h 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 compared to negative control sera [8].

Capri pox double antigen ELISA

Bovine and Ovine samples were examined to detect antibodies against Capri Pox Virus (CPV) by using capripox double Antigen ELISA. The test was utilized according to the manufacturer instructions (ID-vet).

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.

Statistical analysis

Collected data were organized and managed in a Microsoft Excel spreadsheet for analysis by the Statistical Package for Social Sciences (SPSS). The results of statistical analysis was extrapolated for the sample size difference. Also, Risk factors associated with SPP seropositivity were analyzed using descriptive statistics and univariate analysis Chi-square test, receiver operating characteristic was analyze [9].

Results

The overall Sero-Prevalence determined by virus neutralization 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% Different regions had different levels of Sero-Prevalence ranging from 60% in Elkhowi to 98.3% in Dilling [10] **(Table 1).**

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

Table 1: Sero-Prevalence in individual sample localities in the North and South Kordofan States evaluated by virus neutralization.

Individual animal risk factors

Individual animal risk factors such as sex, age and weight were not associated with the Sero-Prevalence of SPP in the Sudan consistently between the different regions, indicating that these factors do not influence SPP infection. However the breed of sheep was significantly associated with the Gharaj breed showing the highest level of Sero-Prevalence of 84.2% **(Figure 2) (Tables 2 and 3).**

Risk factor		VNT		Chi square	df	P-value
		Negative	Positive			
State	N.Kordofan	69(49.3 %)	71(50.7 %)	32.294 a	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 %)	46.910 a	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 %)	38.211 a	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%)	19.029 a	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%)	29.710 a	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%)	34.351 a	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 %)	38.211 a	2	0.001
	Semi arid	38(59.4 %)	26(40.6 %)			
	Sub arid	19(15.8 %)	101(84.2%)			
	Total	84(34 %)	163(66 %)			
	Total	84(34 %)	163(66 %)			
Insect Bites	Yes	39(52 %)	36(48 %)	15.847 a	2	0.001
	No	10(30.3 %)	23(69.7 %)			
	I don't know	35(25.2 %)	104(74.8)			

	Total	84(34 %)	163(66 %)			
Temp ºC	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 %)			
* Significant by univariate analysis using Chi square						

Table 2: Husbandry and ecological risk factors and their association with Sero-Prevalence of sheep pox.

No	Variable		Result		Chi square	df	P-value
			Negative	Positive			
1	Age	Adult	23(69.7%)	10(30.3%)	0.236	1	0.627
2		Young	29(64.4%)	16(35.6%)			
3	Sex	Female	50(64.1%)	28(35.9%)	0.008a	1	0.928
4		Male	2(66.7%)	1(33.3%)			
5	Species	Bovine	39(95.1%)	2(4.9%)	30.881a	1	0.000**
6		Ovine	20(39.2%)	31(60.8%)			
7	Location	Khartoum	39(95.1%)	2(4.9%)	30.881a	1	0.000**
8		Kordofan	20(39.2%)	31(60.8%)			
*significant by using Chi square analysis							

Table 3: Capri pox Double Antigen Elisa for Bovine and Ovine Sera.

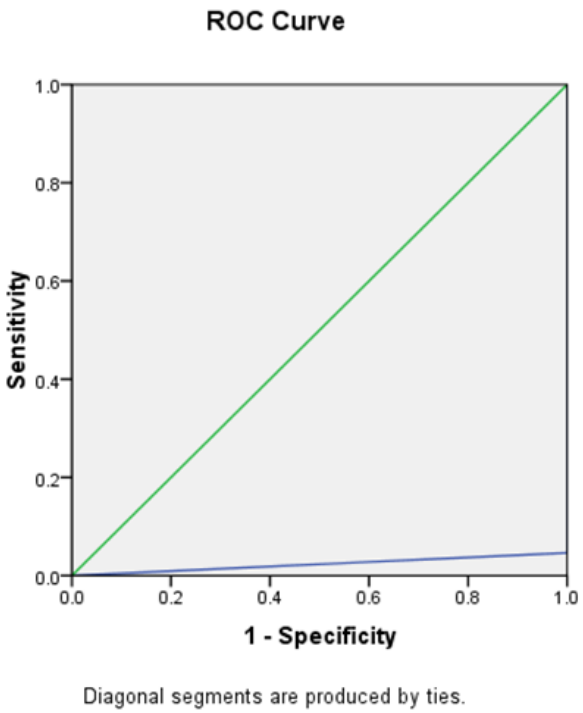

Figure 2: Receiver Operation Characteristic (ROC) to evaluate test performance for ELISA test.

The Age and sex were not significantly associated with seropositivity of Capripox Double Ag ELISA (P>0.05), whereas, species and location were significantly associated with its seropositivity P>0.05 in khartoum and kordofan states.

Other potential risk factors

Out of the 10 investigated putative husbandry and ecological risk factors, seven were found to be significantly (p<0.05) associated with the VNT Sero-Prevalence of SPP. These factors were state, locality, ecotype, mixed herd, herd size, movement pattern and agri-ecological zone. The data revealed that having insect bites decreased the Sero-Prevalence. Temperature and relative humidity factors were found to be not significantly associated with SPP seropositivity (p>0.05) [11].

Questionnaire survey about animal production practices

The questionnaire survey demonstrated that the production system of sheep herders in NKS and SKS was 77% sedentary and 23% nomadic herds. Most of the sheep herders interviewed were found to be aware of SPPV signs in general, with 68% of them were aware of skin lesion, and 13% aware of respiratory signs, while 9% were aware of digestive distress, and 9% that was aware of the complex form of SPPV. Also, herders have experienced SPP occurrence in their flocks, with 59% in the last 2 years, 23% before 5 years, and 18% before 10 years. For vaccination, only 9% of herders had used vaccination against SPPV in their flocks. Quarantine is rarely applied, only 16% of the respondents use quarantine as control measure against SPPV; 16% adopted a mix of strategies including isolation of infected

animals, treatment and restricted movement. Regarding traditional treatment, Acacia species emulsion against SPPV, however, 12% of respondents had claimed that it was as efficient as modern veterinary practices like vaccination and treatment. In regard to the age which sheep are most susceptible to SPPV, 82% were of the opinion that SPPV 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 [12].

Discussion

The overall Sero-Prevalence of SPP in Kordofan region using virus neutralization assay was 73.4%. However, Sero-Prevalence was recorded 5% for lumpy skin disease in Bovine and 62% with 95% specificity sheep pox in Ovine by using Capripox Double Ag Elisa in khartoum and Kordofan, then Reciever Operation Characteristics(ROC) was used to analyze test performance respectively figure. This improvement to the test performance characteristics in comparison to neutralization as a gold standard renders ELISA as a suitable candidate for serological diagnosis for CPV for our laboratory condition in Sudan. The current study revealed that SPPV prevails both in South Kordofan state and in North Kordofan State. Also, a number of risk factors such as sheep breed, geographic region, herd size and movement pattern were found to be significantly associated with SPPV Sero-positivity in the Kordofan region. In comparsion, lumpy skin disease was not prevailing in Khartoum state. It is not surprising that different sheep breeds may be more susceptible to SPPV. Small flock size between 50-100 head was identified as a risk factor. The smaller the flock size was the greater risk SPPV could spread in close flock space. This argument agrees with a report on the outbreak which occurred in 60 goats in a flock in Shambat and Halfaya area (Khartoum North). It was observed in the Kordofan that nomadic herds had a greater Sero-Prevalence of SPPV compared to fixed farms. The increased movement of animals likely contributes to the mixing of flocks increasing the spread of SPPV. The presence of insect bites was found to decrease the Sero-Prevalence of SPPV. However, the role of insect vectors is currently unclear for the transmission of sheep and goat pox and SPPV. It does not require insect vectors for transmission and it is likely that the presence of insect vectors is not a major risk factor for the spread of SPPV in study area. Temperature and relative humidity were not significantly associated with the Sero-positivity of SPPV. However, this is in contrast to lumpy skin disease virus where the disease occurs following rainfall in the wet season when insect vector are present to transmit the disease.

Conclusion

The questionnaire survey revealed that 68% of the respondents were familiar with the skin lesions of SPPV, which agrees with Losos (1986) who reported that the nodular form of SPPV did prevail in the Sudan, as seen in the Rawasi, Bagir and University farm SPPV outbreaks. Fourty 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 SPPV in their flocks. The use of vaccination is the only effective way to deal with SPPV. 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, mostly veterinary services are public sector, means all the services provided by the government including vaccination and treatment are subsidized, but now privatization had surged which let on of the farmer's opinion not to choose vaccines reduces the productivity. Beside that sheep pox vaccine is one of the veterinary biological products that is domestically developed and manufactured which lead to reducing of production cost and being provided at a reasonable price to the farmers. These results indicate that it of outmost importance to educate producers about SPPV and the value of using vaccination to prevent the disease and decrease production losses. Improved multivalent vaccines currently being in development phase. This will help provide a solution for these farmers [13].

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