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ORIGINAL ARTICLE

Seroprevalence of some Infectious transboundry diseases in cattle imported from Sudan to Egypt

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ABSTRACT

Objective: Animal trade has an important role in the economy but in contrast, it causes the spread of infectious diseases overall the world, in particular, the trans-boundary animal diseases. Therefore, the aim of this study is to report the prevalence rate of some transboundary infectious diseases to assess the effectiveness of quarantine measure in the detection of exotic disease and clarify the role of live animal trade in infectious transboundary diseases spread.

Materials and Methods: The study was done on 176 serum samples obtained from cattle imported from Sudan in order to determine the prevalence of foot and mouth disease (FMD), Peste Des Petits Ruminants (PPR), and Infectious Bovine Rhinotracheitis (IBR). Three serological tests were used; Serum neutralization test for FMD, Indirect enzyme-linked immunosorbent assay (i-ELISA) for PPR, and Competitive ELISA for IBR.

Results: The seroprevalence of FMD in tested sera was; 77.27% in the serotype A (A-Iran), 68.18% in the serotype A (A-Africa), 93.82% in the serotype O (O-Pan Asia), and 35.227% in the serotype South African Territories-2 (SAT-2) SAT-2. While the overall seroprevalence of PPR was 49.431% and the IBR was 93.75%.

Conclusion: The result indicates the serious role of live animal trade as "hubs" for infectious diseases spread. Subsequently, the common control measures must be taken to avoid the spread of the diseases through the animal trade; which include screening, surveillance, precautions at borders, and vaccination.

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Introduction

Livestock trade in Africa has an essential role in the national economy, especially in poor communities [1]. On the other hand, cattle trade movements consider seriously as major risks for animal diseases spread, where the live animals and their product are the important vehicles for spreading diseases; especially Africa is a home to numerous major endemic animal diseases [2], in particular, those categorized as "trans-boundary animal diseases" (TADs) [3,4]. TADs are highly contagious diseases of livestock all over the world [5]. Both two types of TADs; emerging diseases and zoonoses have a negative effect on international trade [6]. The important issue is that the majority of Egypt's live cattle for immediate slaughter comes from Africa (mainly Sudan, Ethiopia, and Somalia), therefore, the control of these diseases and to prevent their entrance to the country require effective quarantine system and strict cooperation between different countries [7]. The first step in diseases control is the epidemiological monitoring, which is a preliminary procedure in disease control and eradication. Subsequently, in the current study, three major diseases were selected to be investigated in the veterinary quarries

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to stand on the situation of their prevalence in imported animals and they are; foot and mouth disease (FMD), Peste Des Petits Ruminants (PPR), and Infectious Bovine Rhinotracheitis (IBR). The three diseases were included by Office International des Epizooties in the list of notifiable diseases [8].

FMD is a highly contagious viral disease; it can be considered as a serious and devastating disease on livestock [9]. The contagious nature of FMD virus (FMDV) is an inevitable consequence of many factors, including a large number of susceptible animal, high concentration of virus in animal excretion, low required dose of virus for infection, and the plenty of routes by which the virus can transmit [10]. The disease is caused by FMDV, which belongs to family Picornaviridae; genus Aphthovirus, it has seven serotypes; all of them are immunologically distinct, thereby increasing the burden of the disease [11]. In the African continent, all serotypes already exist, except for Asia-1 [12]. The FMDV carrier state in which the virus can be present be in the oropharyngeal area for long period post infection [13], increases its seriousness [14]. Subsequently, new outbreaks can be initiated by such animal [15]. In Egypt, many of emergence outbreaks probably originating and linked to trade of animals from East Africa [16].

PPR (also termed as goat plague) is a notifiable transboundary disease having a disastrous effect on small sheep and goat, can cause mortality rates which may reach 100% in naive animals [17]; caused by extremely contagious virus, (genus Morbillivirus, family Paramyxoviridae) that is highly linked to Rinderpest (RP) virus [8]. However, the PPR virus (PPRV) affects mainly small ruminant but many others species are considering susceptible to the infection, for example, the virus can infect cattle without any clinical signs but show a seroconversion [18]. Thence can be inferred that the morbilliviruses can switch hosts, with a chance of new ecologic niches created by the eradication of RP [19]. Subsequently, there are conclusive questions about the role of the other host, in particular, whether cattle is a useful sentinel animal of virus entrance and circulation [20]. The disease had become endemic in the majority of Africa and throughout Asia [21], negatively impacting food security, especially in poor communities [22]. There is an assumption that live animal trade moving up to Egypt has a role in the spreading of the infection into north and east Africa [23]. This assumption can be confirmed by a close relation between PPRV IV lineage in Egypt and northern Africa with PPRV lineage initially identified in Sudan [24].

Bovine herpesvirus type 1 (BoHV-1) is a serious virus affecting cattle, causing many symptoms with two common syndromes, respiratory infection; IBR (red-nose) and venereal disease (IBR; infectious pustular vulvovaginitis-IPV) in females or males [25], in addition, there are many other clinical signs, including conjunctivitis, encephalitis and abortions, balanoposthitis, and generalized disease in newborn calves [26]. The virus is a member of the genus Varicello virus in family Herpesviridae [27]. IBR/IPV can act as a barrier to international trade [28]. There are four subtypes of the virus are known: 1.1 and 1.2a (associated with IBR), 1.2b (associated with IPV and infectious balanoposthitis) and 1.3 (encephalitis) [29]. Herpes viruses have become a major subject in virology as a result of widespread infections whose main feature is the establishment of latency [30]. The link between the animal trade and the disease lies in the stressors that are generated during the shipping operations, which can induce reactivation of the latent infection [31]. Subsequently, the virus can switch from latent to lytic infection causing transmission of the disease to contact animals [32].

The aim of this work is to determine the seroprevalence of these serious diseases, as the epidemiologic surveillance is a preliminary and important step in their control, in addition to the focus on the role of livestock trade in their spread, and subsequently make an assessment of the effectiveness of quarantine measure in the detection of exotic diseases.

Materials and Methods

Serum samples

This study was carried out during 2016–2018 at the Laboratories of FMD Department and cell bank, Middle East for Veterinary Vaccines (ME VAC®) Co. One hundred and seventy-six serum samples were collected randomly from apparently healthy cattle imported from Sudan at the time of slaughtering at Veterinary Quarries; 98 samples were collected in Summer season and 78 samples collected in the winter season.

Serum neutralization test for antibodies detection of FMDV serotypes

The sera were tested for detection of protective antibodies against serotypes of FMDV [A (A-Iran05), O (Pan Asia), A (A-Africa), and SAT-2], which were kindly provided by FMD Department, ME VAC®[1] Co. The provided viruses were the fifth passage of the bovine-derived virus on Baby Hamster Kidney-21 (BHK-21). The test applied in the microtiter plate as performed by Golding et al. [33]. Serially diluted (twofold) and heat-inactivated serum (56°C, 30 min) were used against 100 TCID50 FMDV (previously titrated). Fifty microliter of BHK-21 cell suspension was added to previously incubated plates at 37°C in 3%–5% CO_2 incubator for 1 h. Cytopathic effect was read after incubation for 48 h using an inverted microscope. The titers positive cut-off value was 1.2 $\log_{10}$ serum titer (i.e., ≥1/16) [34].

Enzyme-linked immunosorbent assay for detection of antibody against PPRV

Antibodies against PPRV were detected using PPRV Antibody enzyme-linked immunosorbent assay (ELISA) Kit [Shenzhen Lvshiyuan Biotechnology Co (Green Spring)®2 China; Lot NO: 20170301] as described by Balamurugan et al. [35]. The cut off for the positive sample was equal or greater than 0.5 and the cut off for negative samples was less than 0.5.

ELISA for detection of antibody against IBR virus (IBRV)

Antibodies against BoHV-1 were detected using BoHV 1 Test Kits, ELISA BoHV-1gB (Bovichek®, Canada; Lot NO: 156510PL) as previously performed by Cho et al. [36] with little modifications. The cut off for the positive sample was equal or greater than 50% inhibition percentage and the cut off for negative samples was less than 40% inhibition percentage.

Statistical analysis

Statistic analysis of the obtained data was done by using Graph pad prism7 program on bases of serotype and season of samples collection by using: *Chi*-square, Student *t*-test—unpaired, and Fisher exact test). The used *p* value is <0.005.

Results and Discussion

Livestock trade considered as the main reasons for infectious diseases spread between different geographical areas [37]. The effective control of the diseases depends on accurate epidemiologic surveillance [38]. All samples were taken from apparently healthy animals without any signs of infection or lesion for determination of seroprevalence of the selected diseases and clarify the role of live animal trade in their spread.

FMD

FMD has a great economic impact globally [39]; this economic importance comes from its effect on the investment and development of the livestock sector, in addition to export trade opportunities and global food supply [40]. The obtained result revealed that there was a high titer of protective antibodies against different serotypes of FMD: where the serotype A; (A-Iran) had seropositivity 92.85% and 57.69% with mean positive titer 1.67 ± 0.20 and 1.48 ± 0.19 in summer and winter season, respectively, with overall seropositivity result of 77.27%. In statistical terms, there is a highly statistically significant difference in seroprevalence of FMD in two seasons and the serotype A; A-Africa had seropositivity 84.46% and 47.44% with mean positive titer 1.54 ± 0.23 and 1.33 ± 0.21 in summer and winter seasons, respectively, with overall seropositivity result of 68.18%, in statistical terms, there is highly statistically significant difference in seroprevalence of FMD in two seasons. The serotype O (O-Pan Asia) had seropositivity 96.94% and 93.3% with mean positive titer 1.73 ± 0.13 and 1.725 ± 0.16 in summer and winter seasons, respectively, with overall seropositivity of 93.82%, in statistical terms, in contrast to the serotype A, there is no significant variation in seroprevalence of FMD in two seasons. The serotype SAT-2 had seropositivity 52.04% and 14.12% with mean positive titer 1.41 ± 0.21 and 1.34 ± 0.16 in summer and winter seasons, respectively, with overall seropositivity result of 35.227%, in statistical terms, there is a significant in seroprevalence of FMD in two seasons. Statistically, the seropositivity of serotypes O, A, and SAT-2 has a significant difference, as shown in Table 1.

There are many challenges in control of FMD in Africa; one of them is the absence of marker vaccine to backup "Differentiating Infected from Vaccinated Animals" test [41]. Subsequently, the obtained result may indicate the widespread of FMD across Sudan, probably due to the extensive livestock husbandry systems adopted in Sudan, which allows the spread of the virus [42]. Four serotypes of the virus are present in Sudan; (O, A, SAT-1, and SAT-2) [43]. In the current study, the serotype O has the largest percentage of seropositivity 94.88% followed by serotype A 77.72% and finally, the SAT-2 serotype comes in the last with 35.227%. This agrees with the study applied in

Table 1. Serosurveillance results of FMDV serotypes [A (A-Iran), O (O-Pan Asia), SAT-2, and A (A-Africa) in two seasons (summer and winter).

	Summer (*n* = 98)		Winter (*n* = 78)		Significant (*p* < 0.05)
	No of positive samples	Positivity (%)	No of positive samples	Positivity (%)	
Serotype A (A-Iran)	91[a]	92.85	45[a]	57.96	*p* = 0.001**
Serotype O (O-Pan Asia)	95[ab]	96.94	72[b]	92.3	*p* = 0.297
Serotype SAT-2	51[c]	52.04	11[c]	14.12	*p* = 0.001**
Serotype A (A-Africa)	83[a]	84.46	37[a]	47.44	*p* = 0.001**
Significant *p* value < 0.05	*p* value = 0.001**		*p* value = 0.0001**		-

*: $p < 0.05$ (Significant), **: $p < 0.01$ (Significant), $p > 0.05$ (Non-significant).
The numbers followed by same small letter are not statistically different.

South Sudan, whereas the serotype O has the widespread with a major role in yearly outbreaks [44]. Serotype O in Egypt also has the upper hand in all outbreaks till 2005; with exception of 1972, the serotype A was the cause of the outbreak in this year [45]. Also, the result of the study carried out in Libya refers to that the dominance was for serotype O then serotype A [46]. Others studies of seroprevalence of FMD serotypes in Sudan have been reported: the study of prevalence in rat of FMDV in Khartoum state revealed that the antibodies against serotype O are (95%), serotype SAT-2 (80%), and then type A and SAT-1 (57%) [47]. In other study applied in Sudan, the cattle have the prevalence of antibodies to serotype A and O of 78.1% and 69.4%, respectively and antibodies to serotype SAT-2 and SAT-1of 44% and 20.2%, respectively [42].

The current result reveals that there is seasonal variation, with an increase in seroprevalence of FMD in summer season than the winter season, this can be explained by high interaction of animals at grazing area and water spots, which were mostly used by animals brought from different regions in hotter month [47]; with unrestricted high herd mobility during rainy seasons [48].

There are many problems related to the live animal trade in Africa; one of these problems is that the continuous mobility of animals within and across the borders of countries in both term legal and illegal, based on this, the animals which have been imported from certain country, for example, Sudan, they do not necessarily have to be Sudanese origins [49]. Subsequently, the risk of diseases dissemination from different origin operates in both directions, for the importer and exporter countries [5]; Import risks correspond to risks associated with the import of potentially infected animals. Export risks correspond to risks associated with the export of animals to locations where the disease may be present and subsequence contamination of material returning in trade vessels [50].

To link the obtained result and its impact on Egypt, we must know that the importation of live animals from Africa, in particular, from Sudan and Ethiopia has been increased to backing the political relations [51], which can carry the risk of spread of new outbreaks where the cattle recovered from FMD could initiate new outbreaks of the disease, in particular, in endemic countries like Egypt [52]. This can be explained by that among every 20,000 head of imported cattle from Sudan, at confidence levels between 5% and 90% there is a probability of introducing 75 infected animal with a new strain of FMDV or 3.75 infected animal among 1,000 imported animal [40]. This may be the reason that many outbreaks of FMD have been occurred, with the virus remain circulated in vaccinated cattle populations in Egypt as a result of continuous introduction genetically and immunological different serotypes and topotypes [53], for example, most of the current FMD SAT-2 outbreaks reported in Egypt are associated with animals coming from Sudan and Ethiopia [54].

Peste des petits ruminants

Despite the PPRV mainly affects small ruminants [55], the cattle can be infected with no clinical singe and can act as sentinel animal for detection of the disease, especially when the mass vaccination of sheep and goats have been performed [38]. This is depending on that RP vaccination campaigns has been declared in African countries as RP free and using of the RP Vaccine For PPR disease control was stopped [56], this means that the cattle were neither vaccinated against the RP virus nor PPRV, therefore, the seropositivity of the cattle against PPRV is only the result of field infection due to contact with infected (diseased) sheep, vaccinated sheep as a lateral spread, or uncertainly from apparently normal other infected cattle with PPRV, which may be due to that both large and small ruminant shared the same grazing area and watering points [57]. So, the study used the cattle as an indicator for seroprevalence of PPR disease. In the current study, protective antibodies were detected in cattle by ELISA. The obtained result revealed that seroprevalence of PPR in 98 tested samples collected in summer was 57% seropositive (56 positive samples), whilst in 78 tested samples collected in winter was 39.75% seropositive (31 positive samples). The overall seropositivity was 49.431%. Statistically, there is a significant variation in seroprevalence of PPR in two seasons (summer and winter), as shown in Table 2. There are various rates of seropositivity in cattle; in a study applied in Sudan: the protective antibodies against PPRV were found (25.8%) of cattle sera [58]. Also, the seropositivity rate of PPR in Sudan was 67.42% in buffaloes and 41.86% in cattle living with the sheep and goats [57]. Other surveillance performed in Sudan reported that seropositivity of PPR in cattle and camel was 14% and 11.4%, respectively [59].

In northern Tanzania, the antibodies against PPRV were detected in 26.7% of cattle that lived during the 2008 PPR outbreak [38]. It seems obvious that there is variation in prevalence of the disease in different studies, but there are multiple factors that cause these variations including different husbandry system in different geographical regions, climatic factors, and animal's movement in different areas [20]. In addition, the high seropositivity rate obtained in current work may be due to increased contact between large and small animals in areas from which the imported cattle were collected as result of higher population density and sharing the same source of water and pasture [60], also the seasonal variability in the seroprevalence of the disease; where the disease is higher in summer than winter, may be due to constant mobility of animals during rainy seasons (July–October) subsequently increases the chance of exposure to the infection [56]. Ultimately, all the above

Table 2. Seroprevalence of PPR in serum samples of cattle tested by PPRV antibodies detection ELISA kKits.

	No of positive samples	Seropositivity (%)	Significance ($p < 0.05$)
Summer (no: 98)	56	57	$p = 0.023$*
Winter (no: 78)	31	39.74	
Total (no: 176)	87	49.43	

Table 3. Seroprevalence of BoHV-1 in serum samples of cattle tested by BoHV-1 test ELISA kits.

	No of positive samples (no: 98)	Seropositivity (%)	Significance ($p < 0.05$)
Summer (no: 98)	91	92.85	$p = 0.756$
Winter (no: 78)	74	94.87	
Total (no: 176)	165	93. 86	

clearly emphasized proof for the ability of PPRV to infect the cattle under natural conditions without clinical singe [60], although there are no clinical signs in cattle under natural conditions but this is risky in terms of changes of virulence with subsequently PPR can emerge as cattle disease [20]. There are other evidence which increases this possibility as another study which used the polymerase chain reaction assay; PPRV genome can be detected in the nasal excretion of the dogs [61]. Subsequently, this issue urgently necessitates more investigation to determine: the species which only seroconvert without shedding of virus; species with clinical signs and actively shed the infectious virus; and species with inapparent clinical signs but it remains infectious, shedding virus [62]. And the issue which is more important is the virus in its way to adapt to a new host or not [63].

Bovine herpesvirus 1

The IBR disease has an important impact on the international trade of livestock and their products [64]. Based on that, the accurate and specific diagnostic test is necessary in effective control of IBR [30], antibodies against BoHV-1 were investigated by the Blocking ELISA, as ELISA is approved for international trade by the World Organization for Animal Health [64]. The result indicates that the seroprevalence of BoHV-1 infection in 176 tested serum samples was; the seropositivity in Summer and Winter was 92.85% and 94.87%, respectively, the total number of positive samples were 165 with overall 93.75% seropositivity %, with most of the seropositive samples give high inhibition percentage in blocking ELISA range from 80% to 90%. Statistically no significant variation in seroprevalence of IBR in two seasons (summer and winter), as shown in Table 3. There are many studies that revealed highly prevalence rate of infections in cattle with BoHV-1 [65]; wide range of prevalence was found in the study applied in Sudan where the prevalence was 14% and 72% in Northern Kordofan State and Western Kordofan, respectively, this variation can be explained by the differences in husbandry system and/or ecological differences. For example, the higher prevalence rates may be due to high population density in dairy farms which facilitate and increase the disease transmission [66]. In a seroepidemiological study of cattle in Sudan, the seroprevalence rate ranging from 83.33% in West Cordovan to 32% in the River Nile States. In addition, Elhassan et al. [67] carried out a study to determine the seroprevalence of the virus in cattle with reproductive problems in Sudan; the seroprevalence rate of BoHV-1 was 86.8% in infertility cases, 84.3% in aborted cases, and 75% in death after birth. Other study applied on the Coinfections of Sudanese dairy cattle; the seropositivity of BoHV-1 was 84.4% [68]. However, the current result indicates a slightly higher prevalence than other studies but this can be explained by many causes: firstly, this may be due to the reactivation of latent infection as a result of the stress during transportation of animals, where the animal with reactivated virus can shed the virus without clinical disease and infect other contact animals [69], Great majority of animals that are seropositive to BoHV-1 considered latently infected one and can shed the virus when reactivation occur in response to any stressors [27]. Therefore, the serologically positive animals have to be assumed as infected with BoHV-1 [70], the other cause may be due to the collection of cattle before exportation from different areas with variability in the diseases to which they exposed causing continuous circulation of variable infectious agent including BoHV-1, also there is no program for vaccination of cattle against BoHV-1 viruses in Sudan [67].

Conclusion

The current result gives clear evidence that live animal trade has a great role in infectious disease spread, therefore, providing of a effective diagnostic laboratory for rapid and accurate diagnosis in veterinary quarry is necessary as the first step for control measures of infectious animal diseases, furthermore cooperation between the African countries for control strategy is required; the second step, construction of border veterinary quarries where animals are slaughtered directly and are not allowed to enter the depth of the country to avoid the spread of disease. And the most important issue is that; the direct attention of the veterinary authorities must be given to the possible risk pathways associated with importation of livestock cattle from Africa to prevent introduction of infectious diseases of cattle origin as FMD and IBR, in addition, the great attention must be given to cattle's role in other diseases which

do not known as cattle disease as is the case PPR, where the cattle can act as useful sentinel animal for detection of PPRV mixed population and there is an urgent need for further research to detect the main source of the disease in cattle and clarify if cattle are capable of infecting other animals.

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Conflict of Interests

The authors have no conflict of interest.

Authors' contribution

Adel Abdul Azim Fayed gave the main idea of the study and drafted the manuscript. Sahar Hussein Abdalla Hekal carried out the practical work of the study and also contributed to manuscript preparation. Magdy Hassanein Al-Gaabary, Magdy Mahmoud El-Sayed, and Hassan Mohamed Sobhy took part in preparing and critical checking of this manuscript.

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