### Abstract

Aim: to detect and identify the causative agent or agents of the following clinical symptoms which were fever, lack of appetite, salivation, vesication, erosions of the buccal mucosa, nose and feet. The signs vary from mild to severe. The mortality rate of the disease is high. The morbidity rate reaches up to 100%. Sheep also show bloody diarrhea, and rapid respiration. Sheep flock resident in El- Kharje Governorate. Materials: Fifty serum samples and 50 buffy coat samples were collected from Marino sheep flock suffered from high mortalities, fever, lameness, diarrhea, stomatitis and respiratory distress. PrioCHECK FMDV NS (marketable ELISA kit) was used for revealing of the nonstructural antibodies and Liquid phase blocking enzyme immunoassay (LPBE) for identifying the FMD serotype and examined by Competitive enzyme linked immunosorbent assay (cELISA) for detection of PPR antibodies. The buffy coat samples were examined by Immuno-capture ELISA (Ic ELISA) for detection of PPR antigen. Results: By using PrioCHECK FMDV NS: Commercial ELISA kit: 38/50 (76%) of the serum samples were positive to the presence of FMD nonstructural viral proteins. In addition, by using Liquid phase blocking enzyme immunoassay (LPBE) the positive samples were identified as FMD serotype O. Examination of the serum sample by competitive enzyme linked immunosorbent assay (cELISA) for detection of PPR antibodies gave positive results in 32/50 (64%). While the Immuno-capture ELISA (Ic ELISA) identified 32 (64 %) positive reactors for PPR antigen. Conclusion: This study reflected high susceptibility of the imported sheep flocks to the infection with FMD and PPR viruses, which are endemic in the KSA. So the imported flocks that prepared for slaughter must be vaccinated with the used vaccine in KAS in the quarantine for the control of FMD especially when importation occurs from counters that are free from these diseases.

### Keywords

Keywords: FMD, PPR, Marino sheep, virus, KSA.
Outbreak of FMD and PPR in sheep flock imported for immediate slaughter in Riyadh

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Aim: to detect and identify the causative agent or agents of the following clinical symptoms which were fever, lack of appetite, salivation, vesiculation, erosions of the buccal mucosa, nose and feet. The signs vary from child to severe. The mortality rate of the disease is high. The morbidity rate reaches up to 100%. Sheep also show bloody diarrhea, and rapid respiration. Sheep flock resident in El-Kharje Governorate.

Materials: Fifty serum samples and 50 buffy coat samples were collected from Marino sheep flock suffered from high mortalities, fever, lameness, diarrhea, stomatitis and respiratory distress. PrioCHECK® FMDV NS (marketable ELISA kit) was used for revealing of the nonstructural antibodies and Liquid phase blocking enzyme immunoassay (LPBE) for identifying the FMD serotype and examined by Competitive enzyme linked immunosorbent assay (cELISA) for detection of PPR antibodies. The buffy coat samples were examined by Immuno-capture ELISA (Ic ELISA) for detection of PPR antigen.

Results: By using PrioCHECK® FMDV NS: Commercial ELISA kit: 38/50 (76%) of the serum samples were positive to the presence of FMD nonstructural viral proteins. In addition, by using Liquid phase blocking enzyme immunoassay (LPBE) the positive samples were identified as FMD serotype O. Examination of the serum sample by competitive enzyme linked immunosorbent assay (cELISA) for detection of PPR antibodies gave positive results in 32/50 (64%). While the Immuno-capture ELISA (Ic ELISA) identified 32 (64 %) positive reactors for PPR antigen.

Conclusion: This study reflected high susceptibility of the imported sheep flocks to the infection with FMD and PPR viruses, which are endemic in the KSA. So the imported flocks that prepared for slaughter must be vaccinated with the used vaccine in KAS in the quarantine for the control of FMD especially when importation occurs from counters that are free from these diseases.

Keywords: FMD, PPR, Marino sheep, virus, KSA.

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Introduction

Foot and mouth disease (FMD) virus or Aphthus fever virus is a member of Family Picornaviridae [1]. It is highly contagious disease of both wild life and domesticated even-toed animals. Authors confined more than 65 wild animal species that are susceptible to FMD infection [2]. The role of wildlife in maintaining and desemination of the virus to other susceptible wild or domestic ruminants is significant [3]. The causative agent of Foot and mouth disease is a positive, single-stranded RNA virus [4].

Serologically, there are seven known serotypes of the virus known as (O, A, Asia 1, C, SAT 1, SAT 2, and SAT 3) [5]. The most prevalent serotype is type O [4]. The causative agent is excreted in all discharges and secretions of the diseased animals so the virus spreads effectively. Infection occurs through direct and indirect contact with the infected materials [6]. Asia 1 serovype is principally found in Asia, frequently in the Middle East and irregularly in Europe [7]. Airborne infection can occur for a distant of 10 km [8]. This makes complexity in the disease control [8]. Infection with any of the serotype does not give protective immunity against another [9].

Diagnostic vesicles and erosive changes occur in the mouth, nose, nipples and feet. The signs vary from mild to severe, while adult animals generally get better; high mortality in young animals is common [10]. Fever, lack of appetite, salivation, vesiculation, erosions of the buccal mucosa, skin of the interdigital spaces, and coronary bands are characteristics [11, 12]. The mortality rate of the disease is (5%) in adult animals and the morbidity rate of FMD reaches up to 100%. The disease is responsible for serious production losses expressed in low milk production and weight loss due to loss of appetite (vesicles in the mouth). FMD has huge impact on trading of animal and animal products [13]. In addition to death of young animals due to destruction of heart muscles, high costs for curing infected animals those cannot be sold because of emaciation and/or infertile [6].

FMDV is a small RNA naked virus with genome of 8.5-kb. This genome encodes to structural and non-structural proteins (NSPs). Antibodies to both structural and non-structural proteins are detected in infected animals. Whereas antibodies to the non-structural proteins could not be detected in vaccinated animals as we use dead vaccine [14]. In FMD diseased animals, antibodies intended to both the SP and NSPs are present. While vaccinated animal produce humeral immunity to the structural protein only,
consequently, in using examinations that can detect antibodies against NPS we can discriminate diseased animals from vaccinated one and this what can we obtain by using the PrioCHECK® FMDV [15].

3 Pest des Petites ruminants (PPR) has noteworthy obstacle to the animal industry as it causes separate financial misfortunes in sheep and goats farms [16]. PPR virus affected small ruminants (goats and sheep) and induces gastro intestinal inflammation and broncho-pneumonia so the disease is called in some areas pneumo-entritis [17]. The Pest des Petites Ruminants virus is a member of genus morbillivirus, Paramyxoviridae [18]. This genus includes a group of close antigenicity viruses; measles virus, cattle plaque virus and canine distemper [19]. By amino acid sequencing of the (F) protein, all the viral isolates of PPR were classified into four lineages (I, II, III and IV). Lineages III and IV were recorded in Saudi Arabia [20]. PPR is endemic mainly in Africa in addition to the Middle East and the Indian subcontinent [21]. The PPR viral particles are execrated in all affected animal secretions and discharges [22]. Transmission of the disease occurs via droplets infection of infected animals [23].

1 PPR is a transboundary disease so it spread all over the south west of Asia continent in the period between 1993 to 1995 [24]. The disease characterized by high morbidity rate reach to hundred percent and in severe outbreak, mortality can cover the total number of the flock, which perishes economic impact in the developing countries [25].

17 This study aimed to detect and identify the causative agent or agents of these losses.

18 Materials and Methods

Ethical approval: International Animal Ethics Committee and local laws and regulations were considered in applying our experiments.

22 Animals: the present study was performed on a sheep farm resident in El Kharj Governorate in Kingdom of Saudi Arabia KSA. The farm contained 3000 male Marino sheep imported in April 2012 for immediate slaughter from Uruguay. The sheep quarantined and approved that it is free from both FMD and PPR. Instead of going to the slaughterhouse directly, the owner passed 1000 animals to the markets in Riyadh. The rest of the consignment was sent to his farm in El Kharj. The first part was not sold after mixing with the other animals in the markets. The owner gathered all the animals again in the farm. Three days passed and the clinical signs began to appear.
1 The symptoms were fever, stomatitis, difficult breathing, salivation, conjunctivitis, lameness, bloody diarrhea, decumbency and death. The animals suffered from high mortality rate reached to 43.3% (1300 heads) within 2 weeks whereas the morbidity rate was over 90%. The animals under the study were not vaccinated against both foot and mouth disease (FMD) and PPR in the KSA. The country of the animal origin is free from FMD and PPR.

Sampling:

Blood and serum preparation:

8 Fifty blood samples were collected by jugular-vein puncture using vacuum tubes containing EDTA and other tubes free from anticoagulant. Plane tubes were left to clot. We collected the serum samples aseptically in 2 ml tube and stored it at -20 °C until used [26].

11 The samples were collected from the animals that showing clear clinical signs in the form of fever, stomatitis, difficult breathing, salivation, conjunctivitis, lameness, bloody diarrhea and decumbency in addition to apparently normal animals.

14 Buffy coat samples were prepared by centrifugation of the anti-coagulated blood tubes at 2000 rpm for 15 minutes. The buffy coats were collected carefully and separately from the surface of the packed RBCs in 2 ml tube. The collected buffy coat samples stored at -20 °C till used [27].

Detection of FMD NSP antibodies:

18 The PrioCHECK® FMDV NS: Commercial ELISA kit which produced by Prioncs Lelystad B.V. to detects antibodies that release due to the infection with the FMD in the sera of ruminant, camel and pigs against the viral non structural (NSPs) proteins. We followed the manufacture instructions. Test plates were coated with FMDV NSP. 3ABC Specific mAb was enclosed in the kit to bind to NSP. The samples were dispensed in the wells of test plate. Incubation for 1 hour at 37 °C then washing three times is applied. Conjugated mAb with horseradish peroxidase (mAb-HRPO) was added. If the tested serum contain antibodies specific to the NSP, the binding site for the mAb-HRPO will block. After incubation and washing of the plate the chromogen (TMB) substrate is dispensed and incubation at room temperature then stopping of the reaction. At a wave length of 450 nm the developed color was measured optically. The percentage of inhibition (PI) was calculated as follows:
$PI = 100 - (\text{OD test sample/OD450 Neg.}) \times 100.$

Sera with $PI > 50\%$ were scored as positive [28].

**Liquid phase blocking enzyme immunoassay (LPBE) for typing of FMD:**

Commercial LPBE kit produced by FMD World Reference Laboratory (WRL), Pirbright, UK was used for detection of antibodies to foot-and-mouth disease virus. LPBE technique was developed according to Hamblin et.al. [29]. The LPBE was applied according standard operating procedure supplied with the kit. Briefly, the test is based upon specific blocking of liquid phase FMD antigen by antibodies in the test serum sample. ELISA plates are coated with anti-FMD antibody. Sera premixed with different serotypes of FMD antigens are then added to the coated plates. If specific antibodies are present in the test sera, they will block the antigen and prevent it from binding to the coating antibody so no color appears. If there are no specific antibodies in the tested sera then the antigen will be available to be trapped on the plate, this will be detected by a positive color indicating negative test results.

**Competitive enzyme linked immunosorbent assay (cELISA) for diagnosis of PPR:**

Competitive ELISA kit and its protocol provided by IAH (Pirbright Laboratory, UK). The test is depending on the rivalry between the monoclonal antibody against and in the tested serum antibodies for fastening to the H protein antigen [30]. The existence of antibodies in the tested serum sample will prevent the monoclonal antibody (MAb) binding leading to the diminution of expected coloration after the addition of anti-mouse conjugate and substrate–chromogen solution. The manufacturers also recommended the 50% competition cut-off as the positive value for routine testing. Both the negative and positive cut-off values were utilized from the controls of the test procedure. By using Immunoskan reader produced by (Flow laboratories, UK), we read the ELISA plates at 492 nm wave length filter. Calculation of the result was gotten automatically by the aid of an installed software on computer connected to the reader. This software is produced by (FAO/IAEA, Vienna, Austria), and facilitate getting the percentage of inhibition (PI) values directly. The programe converts the Optical Density values to percentage inhibition via this formula:

$$PI\% = 100 - \left(\frac{\text{Mean OD of test wells}}{\text{Mean OD of cma wells}}\right) \times 100.$$
Where OD represents the optical density value and cma points to the monoclonal antibodies (MAb) control. Inhibition values more than 50% were considered positive.

Immuno-capture ELISA (Ic ELISA) for detection of PPR antigen:

The collected buffy coat samples were examined by the Immuno-capture ELISA kit. This kit is capable of detecting the PPR antigen in the buffy coat, nasal sawbs and tissue samples of the supposed animal. Examination was conducted as in accordance with what he has done by Libeau et al., [31] in the Central Veterinary Diagnostic Lab., Ministry of Agriculture, KSA. World Reference Laboratory of Rinderpest and PPR (WRLR/PPR), at PirBright, UK, supplied the kit and ELISA plates. The results were clarified by (OPD) chromogen system and measured at 492 wavelength filter. Calculation of the result was gotten automatically by the aid of an installed software on connected computer to the reader. This software is produced by (FAO/IAEA, Vienna, Austria), and facilitate the identification of the percentage of positive (PP) values. The OD (optical density) values were transformed to PP by via the next formula:

\[ PP = \left[ 100 - \left( \frac{\text{OD control}}{\text{test sample}} \right) \right] \div \left[ \text{Median OD of PPR ref. antigen} \right] \times 100 \]

Samples showed PP > 18% were considered as positive.

Results and Discussion

FMD spread rapidly and causes sever financial loses so it is fundamentally to utilize a recommended very sensitive and precise tests for untimely diagnosis of the disease in integration to identify the causative serotype or serotypes involved in the outbreak [2].

Despite widespread of FMD virus across the world, but there are some countries that are not registered any cases for the disease since decades. Uruguay is one of these countries that are free from foot and mouth disease, as well as peste des petits ruminants disease since 2005 yet. As well as in the last period of the last century it has not registered any cases of the same diseases [32].

The characteristic epidemiological situation of Uruguay encouraged Arab importers to import sheep from it because of the freedom from epidemic diseases as foot and mouth disease and PPR. They are importing sheep for immediate slaughter in Saudi Arabia. Once sheep arrive, they are quarantine, examined
to be sure that there are no antibodies to FMD or PPR and free from any other infectious diseases.

2 In our study, the importer after the release of the sheep from quarantine and prove they are free of foot-and-mouth disease and PPR. Instead of sending sheep for immediate slaughter, the importer sent one-third of the consignment to sheep markets in Riyadh and Al-Ahsa and the rest of the consignment was sent to his private farm in Al-Kharj. Sheep prepared for sale is not sold and were returned to the farm after mixing with native sheep. A week after the collection of the consignment fully in his farm, symptoms and mortality began to appear.

8 Using the Commercial ELISA kit for detection of FMD non-structural proteins antibodies (The PrioCHECK® FMDV NS), 38 samples were positive. Positive samples represent 67 per cent of the total number of samples. The positive sera were examined by LPBE to find out the viral serotype of the virus. The detected viral serotype of FMD was serotype O only. This is the Predominant serotype of the foot-and-mouth disease virus in Saudi Arabia [33].

13 It is of great benefit to use of a liquid phase blocking ELISA (LPBE) in applying FMD control programs. The LPBE is very sensitive and specific so it provides more reliable results. As the test could be applied within the same day, so the advantages of the fast delivery of results are. The method is easy to carry out and does not require extraordinary laboratory conditions like cell culturing.

17 We used competitive ELISA for in the revealing of the PPRV antibodies in this study due to its elevated specificity (99.8%) and high sensitivity (90.5%) if collated with the standard virus neutralization test [34]. If the serum samples contain specific PPRV antibodies, it will prevent the attachment of the monoclonal antibodies to the coated antigen. Thus, addition of the chromogen system will not yield a colour [34]21.

22 By using Competitive enzyme linked immunosorbent assay (cELISA) for diagnosis of PPR to detect the presence of antibodies against the PPR virus, it was found that 32/50 (64 %) of the collected sera were positive. It was found that the mortality rate of PPR in sheep was 34.4% and the prevalence of PPR antibodies was 22.4% in different areas of the Middle East countries [35]. There is a virtual difference between the obtained prevalence of antibodies and that obtained by [35]. In our study, we detected the presence of antibodies in sheep actually during the outbreak. Amplified frequency of the disease may be due to the lack of the experience of the animals to these diseases in the country of origin. Also, low age of
the animals as all animals are less than one year old so it is highly susceptible than adult. Mixing the animals from different origins facilitate the spreading of the diseases. Many factors increase the animal susceptibility to PPRV. These factors includes young age, low maternal immunity intake, The poor nutritional status and drastic climatic conditions.

5 By using Immuno-capture ELISA (Ic ELISA) for detection of PPR antigen in the buffy coat samples, 18/50 (36 %) of the samples showed positive results.

7 On using the cELISA we can verify infected from the uninfected animals within the animal population. The presence of antibodies in the serum samples is only due to active infection not immunization, because the animals come from a country prevents vaccination against foot and mouth disease, or PPR. The presence of antibodies to the two diseases is an indication to the incidence of infection in the Kingdom. IT has been proven that there is no antibodies in sheep blood before it were allowed to get out of the quarantine.

13 There are 17/50 sheep revealed mixed infection with both FMD and PPR and this represent 34% of the tested animals. These animals showed antibodies to FMD serotype (O) and PPR in addition to the PPRV antigen.

Conclusion

17 This study reflected high susceptibility of the imported sheep flocks to the infection with FMD and PPR viruses, which are endemic in the KSA. So the imported flocks that prepared for slaughter must be vaccinated with the used vaccine in KAS for the control of FMD in the quarantine especially when importation occurs from counters that are free from these diseases.

21 Authors’ contributions

MMA and SAG conceived the study, performed the fieldwork, collected the samples, carried out the laboratory work, analyzed the data, drafted the pre published, read and accepted the final script.

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Competing interests

The authors declare that they have no competing interests.

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Table 1: Results of the tested samples with four different Elisa types for the detection FMD antibodies and
serotyping in addition to the detection of both PPR antibodies and antigen.

<table>
<thead>
<tr>
<th>50 samples</th>
<th>FMD</th>
<th>PPR</th>
<th>Mixed infection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NSp</td>
<td>LPBE</td>
<td>cELISA</td>
</tr>
<tr>
<td>Positive</td>
<td>38/50 (76%)</td>
<td>Serotype O</td>
<td>32/50 (64%)</td>
</tr>
<tr>
<td>Negative</td>
<td>12 (24%)</td>
<td>18 (36%)</td>
<td>32 (64%)</td>
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Figure (1) different clinical signs of FMD and PPR diseases.