Molecular and serological survey of some haemoprotozoan, rickettsial and viral diseases of small ruminants from Al-Madinah Al Munawarah, KSA

Taha, H.A.¹,², Shoman, S.A.¹,² and Alhadlag, N.M.¹
¹Taibah University, Faculty of Science, Biology Dept., Al-Madinah Al Munawarah, KSA
²Ain Shams University, Faculty of Science, Cairo, Egypt
*Corresponding author email: hodabada3@yahoo.com
Received 27 July 2014; received in revised form 24 October 2014; accepted 5 November 2014

Abstract. The prevalence of some viral and haemoprotozoan infections in goats and sheep was investigated in the present study. The infections of Anaplasma spp. and Theileria spp. were encountered by PCR and cELISA for the detection of Anaplasma spp. Anaplasma spp. was more prevalent than Theileria spp. with a significant difference (P value<0.05) in both goats and sheep, but, the infection in sheep was more prevalent than goats. Also four viral infections were detected: Rift Valley Fever Virus (RVFV), Bluetongue Virus (BTV), Madina Visna Virus (MVV) and Schmallenberg, (SBV) with an overall prevalence of 56.6%, 40.4%, 36.0%, and 6.8% respectively. The factors related to the infections were also investigated. Detection of the antibodies against examined viruses and Anaplasma spp. indicated the widespread of these infections. So far to our knowledge, this is the first study that estimates the prevalence and distribution of MVV and SBV antibodies in Saudi Arabia. Both RVFV and BTV are widespread in KSA, causing Viremia in 7.2% and 25% respectively of total samples. These findings suggested that parasitic and viral infections are considered as important health risks for sheep and goats. For this reason, the current study introduces invaluable information for different governmental agencies for dealing with infection control measures.

INTRODUCTION

The parasitic, rickettsial and viral infections are the most important diseases that threaten the livestock all over the world. In Saudi Arabia, mutton is considered the meat of choice and under certain religious circumstances (pilgrimage season) huge number of sheep and goats are slaughtered and imported from elsewhere in the Middle East. These animals may carry infections (parasitic or viral). Generally, some rickettsial and blood protozoa, including Theileria and Anaplasma respectively have been previously described in small ruminants (Davidson and Goff, 2001; Zahid et al., 2005). They have been reported as diseases of major economic importance, as they cause heavy losses due to mortality, decreased production and lowered working efficiency of affected animals in the tropics and subtropics of the world (Mehlhorn et al., 1994; Zahid et al., 2005). Currently limited data is available concerning these types of infections in Saudi Arabia. Few studies have been reported mainly on the distribution of Thielria and Anaplasma through tick investigation (Al-Khalifa et al., 1987; ElAzazy et al., 2001; Diab et al., 2006). Blood examination surveys for Anaplasma and other infections have been performed in different regions of Saudi Arabia (Hussein et al., 1991; El-Metenawy, 1999). Previous investigations demonstrated that Blue tongue virus (BTV), rift valley fever virus (RVF), foot and mouth disease virus, Maedi visna virus (MVV), etc. are important viral agents infecting ruminant species (Frederick et al., 1999).

Infections caused by RVFV are characterized by severe disease causing
abortion in livestock, particularly sheep and cattle. Humans in the epidemic region are also at high risk for RVFV infection, potentially resulting in thousands of human cases (Rolin et al., 2013). On September 10-2000, the Ministry of Health in Saudi Arabia began to receive reports of unexplained hemorrhagic fever in humans near the Saudi-Yemeni border, with associated animal deaths and abortions. Patient samples from the outbreak were sent to the Centers for Disease Control and Prevention (CDC), where the laboratory analysis confirmed the cases as being caused by RVFV (CDC, 2000).

Bluetongue (BT) is a viral disease of sheep and occasionally cattle, and is transmitted by biting midges of the genus Culicoides (MacLachlan, 1994). Until the 1940s, this disease was recognized only in Africa, and then following a major epidemic in 1956-1957 in Portugal and Spain, the disease was recognized in the United States of America (USA), the Middle East, Asia and later in Australia (Daniles et al., 2009).

Maedi-visna is an economically important viral disease of sheep that occasionally affects goats (Capucchio et al., 2003; Angelopoulou et al., 2006). The maedi-visna virus (MVV), a lentivirus, infects its hosts for life. Although most infections are subclinical, a minority of animals develops progressive, untreatable disease syndromes, including dyspnea (maedi) or neurologic signs (visna) (Carroza et al., 2010).

Schmallenberg (SBV) virus is a new emerging virus detected for the first time in November 2011 in the town of Schmallenberg, Germany (Hoffman et al., 2012). Since then 9 countries have reported congenital malformation in lambs, calves and goat (Herder et al., 2012). In some areas, SBV cross reacting antibodies has been detected in as high as 100% of the cattle surveyed (Elbers et al., 2012), although the clinical and consequent economic impact of this infection is not completely clear as yet (Daminguez et al., 2012). Phylogenetic analysis revealed that SBV belongs to the genus orthobunyavirus within the Bunyaviridae, a large family comprising hundreds of viruses able to infect a broad range of vertebrate and invertebrate hosts (Frederik et al., 1999).

This study aimed to attract the attention of the prevalence of viral and parasitic infections in sheep and goats in Almadinah Almounaurah where, the accurate surveillance data facilitates understanding the true health status of animal populations and to guide the use of limited animal health resources before the disease becomes entrenched.

MATERIALS AND METHODS

Samples Collection
A total of 312 blood and serum samples were collected from goats and sheep slaughtered in local abattoirs of Almadinah Almounawrah and the veterinary clinics affiliated to the Ministry of Agriculture in Almadinah Almounawrah.

Enzyme Linked immunosorbent assay (ELISA)
Serum samples were examined for Anaplasma spp. infection using the commercially available antibody test kit, cELISA, (VMRD Inc., Pullman, WA, USA) according to the manufacturer’s instructions. Out of the 350 serum samples, 250 were also tested for viral infections using four commercially available ELISA kits (ID Screen Rift Valley Fever Competition Multi-species, ID Screen Bluetongue Competition, ID Screen MVV-CAEV indirect Screening test, ID Screen Schmallenberg virus indirect Multi-species Screening test (ID VET, Innovative Diagnostics, Grabeis, France). The kits are based on the detection of antibodies. The protocol provided by the manufacture was strictly followed. All samples were analyzed and calculated with an automated ELISA reader at 450 nm (SIRIO S Elisa Reader, Indonesia).

DNA Extraction
It was performed on blood samples using G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Korea) according to the manufacture’s instructions.
Polymerase Chain Reaction (PCR)  
Polymerase chain reaction analysis was performed to detect *Anaplasma phagocytophthilum*, *A. ovis*, *Theileria lestoquardi* and *T. ovis* using Bio in Gentech Veterinary PCR Kits (Concepcion, Chile) including specific primers for each species. On the other hand, 250 samples were used for viral detection: the MVV DNA fragment was amplified using Taq PCR core kit Quick start protocol (QIA-gen, California, USA).

RNA isolation  
Plasma and serum samples were cleared by centrifugation in a tabletop centrifuge at 10,000xg for 10 min. Viral RNA was prepared from 140 µl of the cell free fluid by using the QIAamp Viral RNA kit spin protocol (QIAamp, GmHb, Germany) without modification. RNA was eluted in 60 µl then subjected to RT-PCR.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)  
A single tube RT-PCR amplification was carried out using one step RT-PCR Quick start protocol (QIA-gen, California, USA). All PCR amplifications were carried out in a 25µl volume per tube. Conversion of RNA to CDNA followed by amplification of the DNA was carried out with Taq PCR core Kit (QIA-Gen, California, USA). The cDNA was amplified at a region that is specific to virus using primers that are selected from the sequence of the virus nucleic acid. The primer sequences used in this study were as follows: RVV was amplified by RVF 5'-CTGTCTGGCACAGCATTGAT-3' and RVR 5'-CACATTGAACACCCACACC-3' (Salim et al., 2010), BTV; BTF5'-AGTTCTCTAGTTGGCACACACC-3' and BTR 5'-TTCACGCTTTCAACCATCC-3' (Shad et al., 1997), MVV; MV15'-AAGTATGAAACAAGGACTAC-3' and MV25'-ATGTCTGTGTTTTTCCACAAT-3' (Zanoni et al., 1991), SBV: ScF 5'-TTGGCCAATTATAAGGACCTAC-3' and ScR 5'-TCAGGATCGCAAAATTTAAGAACC-3' (DeRegge et al., 2012).

Statistical analysis  
Statistical analysis of data were performed using a statistical software program (SPSS for window version 17.0 SPSS Inc. Chicago, USA).

RESULTS  
To investigate *Anaplasma* infection, two diagnostic techniques were performed, cELISA and PCR. However, only one assay, PCR, was used for *Theileria* spp. detection. Based on the PCR assay, four species were identified, two belonging to *Anaplasma* (*A. ovis* and *A. phagocytophylatum*) and the other belonging to *Theileria* (*T. ovis* and *T. lestoquardi*). Comparing the infection rate of *Anaplasma* and *Theileria* in both sheep and goats, it was observed that *Anaplasma* spp. were more significantly prevalent (45.5% and 40.4%) than *Theileria* spp. (23.8% and 22.8%) in sheep and goats respectively (P<0.05). Also, the infection rate in sheep was higher than goats but with no significant difference (Pvalue >0.05).

The associated factors with the positively infected samples are shown in Table 1. Based on cELISA test, the infection rate of *Anaplasma* spp. in sheep (49.2%) was higher than goats (44.7%) with no significant difference (P>0.05). Regardless of type of host and based on the gender, it was observed that the infections in males were more prevalent than in females with no significant differences (Pvalue >0.0.5). However, *T. ovis* was significantly more prevalent in males than females (Pvalue <0.05, Chi squarevalue=3.9). Also, the age of the host played a role in the infection rates in the four species, where the group with age ranged from 2m<2y had significantly higher rates of infections with *A. phagocytophylatum* (Pvalue <0.05) whereas the other three species were more prevalent without a significant variation. It should be mentioned that the infection rates in the imported sheep and goats were higher than local ones with a significant difference (Pvalue <0.05).

Concerning the viral investigations, the prevalence of four viruses in goat and sheep samples using ELISA and PCR is presented in Table 2. Based on gender, the results revealed that infection by RVFV was more frequent in female goats and sheep than males...
Table 1. Seroprevalence of *Theileria* spp. and *Anaplasma* spp. Infections in both goats and sheep based on demographic categories

<table>
<thead>
<tr>
<th>Host</th>
<th>Suggested factors</th>
<th>Parasites</th>
<th>Anaplasma spp. (ELISA)</th>
<th>44.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>A. ovis</em></td>
<td>No. of +ve samples</td>
<td>% of infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. phagocytophilum</em></td>
<td>No. of +ve samples</td>
<td>% of infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. ovis</em></td>
<td>No. of +ve samples</td>
<td>% of infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. lestoquardi</em></td>
<td>No. of +ve samples</td>
<td>% of infection</td>
</tr>
<tr>
<td>Goat (123)</td>
<td>Gender</td>
<td>Male (65)</td>
<td>39 (60%)</td>
<td>19 (29.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female (58)</td>
<td>16 (27.6%)</td>
<td>11 (18.9%)</td>
</tr>
<tr>
<td><strong>Age groups</strong></td>
<td>(1) 2m &lt; 2y (44)</td>
<td></td>
<td>26 (59.1%)</td>
<td>13 (29.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) 2y &lt; 4y (60)</td>
<td>25 (41.7%)</td>
<td>13 (21.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) 4 &lt; 6y (18)</td>
<td>4 (21.1%)</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td>Locality</td>
<td>Local (82)</td>
<td></td>
<td>19 (23.1%)</td>
<td>14 (17%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imported (41)</td>
<td>36 (87.8%)</td>
<td>16 (39%)</td>
</tr>
<tr>
<td>Sheep (189)</td>
<td>Gender</td>
<td>Male (128)</td>
<td>70 (44.3%)</td>
<td>35 (27.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female (61)</td>
<td>23 (37.7%)</td>
<td>14 (22.9%)</td>
</tr>
<tr>
<td><strong>Age groups</strong></td>
<td>(1) 2m &lt; 2y (84)</td>
<td></td>
<td>52 (61.1%)</td>
<td>26 (30.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) 2y &lt; 4y (89)</td>
<td>39 (43.8%)</td>
<td>23 (25.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) 4 &lt; 6y (16)</td>
<td>2 (12.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Locality</td>
<td>Local (107)</td>
<td></td>
<td>34 (31.7%)</td>
<td>19 (17.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imported (82)</td>
<td>59 (71.1%)</td>
<td>30 (36.6%)</td>
</tr>
</tbody>
</table>

*Significant difference in the rate of infections between males and females P_value<0.05, Chi^2_value=3.91
**Significant variation among the three age groups P_value<0.05, Chi^2_value=6.94

with a statistically significant difference (P_value=0.045) in consequence, the rate of infection is largely affected by the gender (Eta=0.159). Similarly the gender has a large effect on infection by BTV (Eta=0.267) where the infection of male goats (64.1%) was lower than female (71.7%) but infection of male sheep (67.2%) was higher than females (42.1%) with highly significant difference (P_value<0.001). In respect with MVV and SBV infections, it was observed that female goats and sheep more frequently infected than males with no significant difference (P_value >0.05). Also, in RVFV and BTV infections were more prevalent in males than females with a significant difference (P_value <0.05).

The same Table 2 illustrates also the age-related prevalence of four viruses in goat and sheep samples. Of a total 103 goat samples there was a difference in virus infections among the three age groups. The detection of RVFV and BTV in age group 1 (2m>2y) of goats (65.7% and 71.4%, respectively) more than the other groups 2 and 3 (59.5% & 47.6% and 65.9% & 61.9% respectively). While the detection of MVV in the age group 3 (4y>6y) was 66.6% more than in the age groups 1 and 2 (31.4% and 31.9% respectively). About SBV infections it was equal in age groups 1 and 3 (14.2%) and more than age group 2 (31.4% and 31.9% respectively). About SBV infections it was equal in age groups 1 and 3 (14.2%) and more than age group 2 (6.3%). Of a total 147 sheep samples the prevalence of RVFV, MVV, and SBV infections were relatively higher in the age group 3 than age group 1&2 (Table 2) while, BTV infection slightly more in the age group 2 (68.6%) than in the age group 3 (65.0%). Statistically it was found the differences between the age and type of viral infections not significant where P_value<0.05.
Table 2. Seroprevalence of viral infections in sheep and goat populations based on demographic categories

<table>
<thead>
<tr>
<th>Host</th>
<th>Gender</th>
<th>Categories</th>
<th>RVFV ELISA</th>
<th>BTV PCR</th>
<th>MVV ELISA</th>
<th>SBV PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>Male</td>
<td>(64)</td>
<td>36(56.2%)*</td>
<td>29(57.6%)*</td>
<td>23(44.6%)</td>
<td>15(22.2%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>(39)</td>
<td>25(64.1%)</td>
<td>28(71.4%)*</td>
<td>18(46.1%)</td>
<td>3(7.7%)</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1) 2m &lt; 2y</td>
<td>(35)</td>
<td>23(65.7%)</td>
<td>25(71.4%)</td>
<td>11(31.4%)</td>
<td>5(14.2%)</td>
</tr>
<tr>
<td></td>
<td>(2) 2y &lt; 4y</td>
<td>(47)</td>
<td>28(59.5%)</td>
<td>31(65.9%)</td>
<td>15(31.9%)</td>
<td>3(6.4%)</td>
</tr>
<tr>
<td></td>
<td>(3) 4 &lt; 6y (21)</td>
<td></td>
<td>10(47.6%)</td>
<td>13(61.9%)</td>
<td>14(66.6%)</td>
<td>3(14.2%)</td>
</tr>
<tr>
<td>Locality</td>
<td>Local</td>
<td>(62)</td>
<td>38(61.2%)</td>
<td>39(62.9%)</td>
<td>27(43.5%)</td>
<td>9(14.5%)</td>
</tr>
<tr>
<td></td>
<td>Imported</td>
<td>(41)</td>
<td>23(56.1%)</td>
<td>30(73.1%)</td>
<td>13(31.7%)</td>
<td>2(4.8%)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Male</td>
<td>(119)</td>
<td>72(60.5%)*</td>
<td>80(67.2%)*</td>
<td>34(28.5%)</td>
<td>9(7.6%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>(38)</td>
<td>25(68.9%)</td>
<td>16(75.7%)*</td>
<td>10(52.6%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1) 2m &lt; 2y</td>
<td>(41)</td>
<td>26(63.4%)</td>
<td>24(58.5%)</td>
<td>9(21.9%)</td>
<td>2(4.9%)</td>
</tr>
<tr>
<td></td>
<td>(2) 2y &lt; 4y</td>
<td>(85)</td>
<td>55(65.9%)</td>
<td>59(68.6%)</td>
<td>29(35.7%)</td>
<td>7(8.1%)</td>
</tr>
<tr>
<td></td>
<td>(3) 4 &lt; 6y (20)</td>
<td></td>
<td>16(80%)</td>
<td>13(65%)</td>
<td>12(60%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Locality</td>
<td>Local</td>
<td>(64)</td>
<td>50(80.6%)</td>
<td>42(65.6%)</td>
<td>32(50%)</td>
<td>6(4.8%)</td>
</tr>
<tr>
<td></td>
<td>Imported</td>
<td>(83)</td>
<td>47(56.6%)</td>
<td>54(65.1%)</td>
<td>18(21.7%)</td>
<td>3(3.6%)</td>
</tr>
</tbody>
</table>

* Means the differences is significant and P_value>0.05
- For RVFV Chi² 0.035, P_value 0.04, Eta_value 0.15
- For BTV Chi² 0.001, P_value 0.001, Eta_value 0.26

According to the locality of the hosts, it was revealed that viral infections of local goat and sheep by RVFV (61.2% and 80.6%), MVV (43.5% and 50.0%), and SBV (14.5% and 4.68%) were more than imported ones (56.0% and 56.6% for RVFV, 31.7% and 21.7% for MVV, 4.87% and 3.61% for SBV respectively). While infection by BTV more in imported goat (73.1%) than local ones (62.9%) and relatively equal between imported and local sheep (65.6% & 65.1%) and all differences not statistically significant (P_value<0.05).

Using another sensitive and specific technique (RT-PCR for RVFV, BTV, and SBV & PCR for MVV), the positive results for both RVFV and BTV were only encountered (as shown in Table 2) where the other two viruses (MVV and SBV) were not detected at all by PCR.

Based on the same categories, it was found the RVFV has been detected with high frequency in male of both goat and sheep samples 15 out of 64 (23.4%) for male goat and 3 out of 39 (7.7%) for female goat, 9 out 119 (7.56%) for male sheep and 0 out of 38(0%) for female ones. About BTV infection 30 out of 64 (46.8%) for male goat and 6 out of 39 (15.3%) for female goat, 49 out 119 (41.1%) for male sheep and 5 out of 38 (17.8%) for female ones and all differences with P_value<0.05.

Using RT-PCR, the distribution of the two viruses among various age groups of animals was also studied. The results in Table 2 represent the majority of goats (27.6%) and sheep (8.13%) within the age group 2 (2y>4y) were infected by RVFV. In respect with BTV infection, it was varied between goat and sheep where, within goat samples the high frequent infection was detected in the age group 2 (44.6%) while within sheep samples the relative frequency of BTV infection was detected in the age group 1 (39.0%) in comparison to other age groups. Also, Table
Table 3. Prevalence of viral infections using ELISA and PCR according to the source of Samples collection

<table>
<thead>
<tr>
<th>Source</th>
<th>Types of animal</th>
<th>ELISA</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinary</td>
<td>Goat (33)</td>
<td>23(69.6%)</td>
<td>2(6.0%)</td>
</tr>
<tr>
<td>Clinic (60)</td>
<td>Sheep (27)</td>
<td>25(92.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Slaughter</td>
<td>Goat (70)</td>
<td>38(54.2%)</td>
<td>16(22.8%)</td>
</tr>
<tr>
<td>house (190)</td>
<td>Sheep (120)</td>
<td>72(60.0%)</td>
<td>9(7.5%)</td>
</tr>
<tr>
<td>Veterinary</td>
<td>Goat (33)</td>
<td>23(69.6%)</td>
<td>2(6.0%)</td>
</tr>
<tr>
<td>Clinic (60)</td>
<td>Sheep (27)</td>
<td>25(92.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Slaughter</td>
<td>Goat (70)</td>
<td>38(54.2%)</td>
<td>16(22.8%)</td>
</tr>
<tr>
<td>house (190)</td>
<td>Sheep (120)</td>
<td>72(60.0%)</td>
<td>9(7.5%)</td>
</tr>
</tbody>
</table>

2 shows the prevalence of infection by the two viruses in goat and sheep samples according to their localities (local or imported). It was found that BTV infection detected with high frequency in imported goat 43.9% relative to 29.0% in local ones and in imported sheep 42.1% relative to 29.6% in local ones. About RVFV infection, it was detected with high frequency in imported goat 24.3% relative to 12.9% in local ones while in sheep samples detected with high frequency in local sheep (9.37%) relative to imported ones (3.61%). Statistically, all differences between samples concerning age and source of animal not significant where P<0.05.

Based on the source of samples collection (Veterinary clinic & Slaughterhouse), the Investigation using ELISA indicated a high prevalence of the four viruses in samples collected from the Veterinary clinic than Slaughterhouse (Table 3). But it varied according to the type of animal where RVFV and MVV infections detected in sheep (92.5 & 48.1%, respectively) were more prevalent than goats (69.6% & 42.4% respectively). Statistically, the differences in RVFV and MVV infections were not significant where P<0.05. Within the same Veterinary clinic samples, infections by BTV and SBV were prevalent in goats (72.7% & 27.2%, respectively) more than sheep (55.5% & 7.4%, respectively) and these differences were statistically significant (P<0.05).

The prevalence of both RVFV and BTV infections was varied using RT-PCR than using ELISA where they were more distributed in slaughter house samples than veterinary clinic ones and their frequent were in goats (22.8% & 47.1%, respectively) than in sheep (7.5% & 43.3% respectively) and statistically these differences were not significant (Table 3). The two techniques (ELISA & RT-PCR) were actually expressing two different types of infections. So, the prevalence of the RVFV and BTV was studied according to each technique (Table 4). The results revealed that all viruses under study were detected by...
ELISA more than RT-PCR where 140 (56.0%) RVFV positive samples were detected using ELISA, while using RT-PCR the number of positive samples were only 18 samples (7.2%). In respect with BTV, it was detected using RT-PCR with a higher frequency (25.6%) than RVFV (7.2%) but still less than the detection by ELISA (40.4%). Totally, 18 samples shared in positivity using ELISA & RT-PCR for RVFV infection, 64 samples shared in positivity using ELISA & RT-PCR for BTV infection. About MVV infection results using ELISA 90 out of 250 (36%) was detected and for SBV infections, 17 out of 250 (6.8%) was also detected in all samples. In the same manner, the detection of Anaplasma spp. by cELISA produced more prevalent positively infected samples (47.9%) than PCR test (43.2%). However, PCR assays discriminated between the two species of Anaplasma.

The number of single, double, triple and quadruple infections with parasites and/or viruses in both sheep and goats is shown in Fig. 1. The observed results in the mentioned Figure 1 revealed that the single infection was the most common, followed by double, triple while the least number of infected samples was noticed in quadruple infections.

On the other hand, based on PCR analysis, comparing the results of viral and parasitic detections in about 200 samples, it was observed that a 2% (4/200) of the examined samples have mixed infections including the four parasitic species and viral species (data not shown). However, 9% (18/200) of the examined samples were concurrently infected with Theileria spp., BT and RV beside 9.5% (19/200) of the examined samples were concurrently infected with Anaplasma spp., BT and RV.

**DISCUSSION**

A screening of the earlier literatures concerning with the viral and protozoan infections in KSA, it was noticed that no data are available investigating the case in Al-Madinah therefore, the current work is considered as the first study carried out in Al-Madinah to gain an insight situation of these infections. The present study was designed to determine the prevalence of parasitic and viral infections in local and imported sheep and goats slaughtered in Medina slaughterhouse and that received at Veterinary clinics affiliated to the Ministry of Agriculture in Al-Madinah Al Munawarah.

In the present study, it has been found that the overall infection rates of Anaplasma spp. and Theileria spp. in sheep were 45.6% and 23.4%, respectively included A. phagocytophilum, A. ovis, T. ovis and T. Lestoquardi. While, goats have been infected
by *Anaplasma* spp and *Theileria* spp. in 40.7% and 22.8% of the examined samples respectively.

In other parts of Saudi Arabia, there have been several studies investigating restricted distribution of *T. ovis* and *T. hirci* as well as *A. ovis* (Hussein et al., 1991; El-Metenawy, 1999; ElAzazy et al., 2001; El-Bahy et al., 2008). According to Al-khalifa et al. (2009), *A. marginale* and *A. ovis* were recorded, for the first time, in raising animals in the Kingdom. While, in the present study, it is the first record of *A. phagocytophilum* in the area of Al-Medinah in addition to *A. ovis*.

In accordance with Altay et al. (2005), (2007) and Durrani et al. (2012), it has been found that *Theileria* spp. and *Anaplasma* spp. were more prevalent in sheep than goats with a significant difference (P<0.05). Also, some authors accepted that goats show significant resistance to the disease in comparison with sheep (El-Hussein et al., 1998; Naz et al., 2012).

Studies from other countries have also shown that infection in sheep is more common than goats (Inci et al., 2010; Altay et al., 2012). Nevertheless, there is an insufficient knowledge of the relative susceptibility of sheep and goats and of various breeds of each species. In the current study, the prevalence of *Anaplasma* and *Theileria* spp. in sheep and goats has been influenced by age and sex. The males were more prevalent than females in all age groups, regardless of host type with a significant difference (P<0.05).

Similar finding (Naz et al., 2012) revealed that the prevalence of *Theileria* in sheep has been influenced by age and sex. The parasites were more prevalent in males than females in sheep, while gender was not found to be a risk factor in goat.

In contrast, Rehman et al. (2010) and Bahrami et al. (2013a) reported that the prevalence of *Theileria* spp. in females was more than males. When we turn our attention to age factor in both goats and sheep, the rate of infection was more prevalent in age group one (2m<2y) than group two (2y<4y) and age group three (4y<6y) with a significant difference (p<0.05). However, Naz et al. (2012) investigated that the parasite was equally distributed (p>0.05) in young and adult goats, while they were more prevalent in young’s than adult sheep without significant difference (p>0.05). On the other hand, previous studies (Jianxun & Yin, 1997; Bahrami et al., 2013b) reported higher prevalence rate in lambs compared with adults. However, Durrani et al. (2011) did not observe any effect of gender and age on the occurrence of *Theileria* in sheep and goat. Moreover, *Theileria* infection was found to be 10.6% and 14.05% in lambs and adults, respectively. In general, the variation of results in comparison with other different studies may be related to the variation in the strain of parasite, the vector, breed and management of animals as well as the season of the year during the study.

For viral investigation 200-250 blood samples were collected and investigated by ELISA and PCR to detect both antibodies and antigen responses respectively. Detection of the antibodies against all investigated viruses in all groups indicates the wide distribution of this infection. The overall seroprevalence of RVFV, BTV, MVV, and SBV was found to be 56.6%, 40.4%, 36.0%, and 6.8% respectively.

Seroepidemological studies of MVV in sheep have been reported before in many countries (Preziuo et al., 2010; Lipecka et al., 2010) using the serological tests, and ELISA has been the most sensitive one (De Andresa et al., 2005). Animal species are the most important factor that effect on susceptibility of animal to viral infection. MVV is a dangerous viral disease infect sheep and occasionally infects goat in contrast to its closely related virus Caprine Arthritis Encephalitis Virus (CAEV) which found most often in goat (Pisoni et al., 2007). CAEV has been recently detected in Saudi Arabia at Qassim (Al-Dubaib, 2014). In this Study MVV was detected in Al-Madina Almunawarah (first record) in sheep as well as goats.

SBV as new emerging arthropod-borne virus is widely distributed in northern Europe countries and recorded as a novel viral disease of cattle, sheep, and goat (Elbers et al., 2012). SBV antibodies like MVV were also as a first time detected in this investigation in different blood samples of sheep and goat.
in Al-Madina Almunawarah (regardless the redundancy of MVV and SBV in tested samples). Both viruses (MVV and SBV) were similar in their distribution among goat and sheep samples. Whereas, seropositivity more prevalent in sheep in relative to goats, in females more than males of both goat and sheep, in age group 1 and 3 for goat more than group 2, in age group 3 for sheep more than group 1 and 2, and in local goat and sheep more than imported ones.

Actually, the emergence of these two viruses in Saudi Arabia probably may be attributed to a combination of many factors including climate and ecological changes, commercial exchange, changes in trading and commercial policies, all these factors created optimal conditions for the influx of infected vertebrate hosts and invertebrate vectors over wide geographical areas (Gale et al., 2009), especially for this country which forced to import huge number of livestock.

Moreover, susceptibility of species more than another as previously mentioned, significant and non significant data might be for introducing certain viral strains infects particular species or prefer one species rather than the other according to breed susceptibility (Yesiblag and Gungor, 2009).

RVFV and BTV are two important epidemic viruses exhibited more prevalence of their antibodies (ELISA data) and their antigens (RT-PCR data) in this study. Both techniques were concerned with two types of infections (late infection and early infection, respectively) so, their distribution was varied among different samples.

It was observed that the viral antibodies detected in female goat and sheep, their frequency in age group 1&3 more than the age group 2, additionally these viral antibodies detected in local animals more than imported. Although, viral antigens using RT-PCR detected in male goat and sheep, their frequency in the age group 2 more than age group 1&3 additionally, these viral antigens detected in imported animals more than local. RT-PCR for detection of the early viral infection is sensitive and important technique in pre-screening for field investigation to predict the peak of virus viremia as reported for RVFV (Shad et al., 1997) and BTV (Zanoni et al., 1991). They recommended this technique during the epidemiological surveys. RVFV previously reported in Saudi Arabia on both human and animal levels. Madani et al. (2003) studied the prevalence of RVFV in the people of southwestern Saudi Arabia and attributed the high prevalence to the introducing of infected imported livestock from Africa or via wind borne infected mosquitoes particularly the climate conditions have promoted sufficient vector populations. Another study was conducted in Yemen and confirmed the relation between human, livestock, and vector (Shoemaker et al., 2002). A recent study on RVFV in Netherlands developed a certain mathematical model to evaluate the transmission potential of RVFV among livestock and concluded the importance to determine the vector competence of the mosquito species for RVFV and its host preference (Fischer et al., 2013).

Bluetongue antibodies are widespread in ruminants in Saudi Arabia (Hafez and Taylor, 1985; Abu-Elzein et al., 1998). Moreover, new stereotypes were introduced into the kingdom. At Al-Asha (eastern Saudi Arabia) a wave of abortion, stillbirth and deformities in sheep occurred in the summer (May to October) and this time showed maximum abundance of Culicoides midges vector finally it was concluded that the outbreak was caused by a newly introduced serotype which was expected in this country import sheep, goat and cattle (Housawi et al., 2004). Another late study detected the seropositivity of BTV among sheep, goat, cattle and camels in the different districts of the kingdom which indicated to the distribution of infection all over the country and attributed this prevalence in this country into the uncontrolled movement and transportation of animals from one region to another (Yousef et al., 2012).

Acknowledgment. The authors are grateful to Deanship of Scientific Research, Taibah University, Al-Madinah Al Munawarah, KSA, for financially supporting this study, Project no. 3089.
REFERENCES


