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Sero-prevalence of Bovine Herpesvirus-1 and Its Associated Risk Factors in District Sheikhupura, Punjab Province

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BoHV-1, Sero-prevalence, Pakistan, Risk factors

ABSTRACT
Bovine herpesvirus-1 (BoHV-1) is globally circulated bovine virus of genital and respiratory importance. The present study was planned to investigate the sero-prevalence of BoHV-1 and its associated risk factors in District Sheikhupura, Punjab, Pakistan. The sample size was calculated using Raosoft sample size calculator. A total of 250 animals were sampled from herds of different sizes for sampling through convenient sampling technique. A questionnaire having variables related to animal species, age, herd size, parity, breeding method and housing management was filled at sample collection time. The serum samples were processed by indirect ELISA to detect antibodies against BoHV-1. The overall sero-prevalence was 68.40%. Post pubertal animals (>3yrs) contributing more to disease occurrence. High sero-positivity was observed in buffalo (73.12%) as compared to cattle (60%) moreover, the sero-prevalence is higher in animals of greater size herds. Parity status is in agreement of strong correlation with BoHV-1 infection. Evidently, positivity rate was higher in animals bred with artificial insemination as compare to natural breeding method.

Dairy products contribute greatly towards our national gross domestic product (GDP) by foreign exchange (Rehman et al., 2017). In Dairy sector, severe consequences are faced by livestock farmers because of infectious diseases but major consequences are faced by viral source of infection through reduction in milk production, abortion, infertility and calf death (Givens and Marley, 2008). Bovine herpes virus-1(BoHV-1) is one of abortion causing virus that prevalent in Pakistan. BoHV-1 belongs to the family Herpesviridae which is further subdivided. Clinically, BoHV-1 causes infectious diseases in bovine as infectious balanoposthitis (IBP) in bulls and infectious bovine rhinotrachetis (IBR) in dairy herd. Another important disease in cows is infectious pustular vulvovaginitis (IVP) which is also caused by BoHV-1 (Hammad-Ur-Rehman, 2021). IBR leads to conjunctivitis, rhinitis and necrosis of nasal mucous membrane and abortions in pregnant animals. BoHV-1 infections causing metritis and early embryonic fetal deaths lead to severe economic losses worth of millions of dollars globally. The total estimate of loss was approximately US$ 379 per cow (Queiroz-Castro et al., 2019).

Several studies have been conducted to find out the sero-prevalence of BoHV-1 in different parts of the Pakistan. These studies are only conducted at farm level. However, no study so far conducted to check the prevalence of the BoHV-1 at the district level, which indicates a need of the BoHV-1 study at district level so that effective policy can be made to increase the profitability of the farmers by reducing the production losses. Keeping in view the reproductive and productive importance, present study is designed to find out the sero-prevalence of BoHV-1 in densely populated area of district Sheikhupura with identifying potential risk factors. Results help to make effective control strategy and eradication programs for BoHV-1 to enhance trade of Pakistan and export of animals to foreign countries.

Materials and methods
This study was planned on large ruminants of district Sheikhupura. It is 16th largest city of Pakistan by population, located in northwest of Lahore. It is track down on world map with latitude 31, 42°59.9796”N and
longitude 73, 59°6.0828’E with variation in climate and average rainfall is approx. 635mm.

A total of 250 (number calculation using Raosoft sample size calculator 160 buffalos and 90 from cattle population) samples were collected randomly from different tehsils of study area in a way to reach maximum UCs of district to depict best picture of sero-prevalence. Brief data was collected on questionnaire during sample collection. Serum was separated from the collected blood samples and stored at -20°C for further processing and analysis. The serum samples were tested by indirect ELISA kit - ID Screen® IBR gE competition ELISA (ID. Vet, Grables, France) following manufacturers instructions.

Descriptive statistics was used to analyze results and to find potential risk factors against BoHV-1 antibodies in herd. Chi-square test was performed with help of SPSS (version 22) and statistically \( p \)-value (< 0.05) was considered significant.

Results

The study revealed overall 68.5% (171/250) sero-positivity of BoHV-1 antibodies in district Sheikhupura. The analysis of contributing risk factors based on chi-square test helped to find the potential risk factors in herds of Sheikhupura district associated to sero-positivity based on prevalence rate and \( p \)-value (<0.05).

In host range, BoHV-1 antibodies were detected more in buffalo (73.125%) population in comparison to cattle (60%). History of animal having abortion was found to be a key risk factor in sero-prevalence of BoHV-1 with 88.27%. As far as age are concerned, cluster with >3 years age of animals having more sero-prevalence than a cluster with <3 yrs age of animals, 71.78% and 54.16%, respectively. In respect of parity status as animals in 0-2, 3-4, 5-6 lactation indicating 52.45%, 72.84% and 76.31% sero-prevalence, respectively with \( p \)-value <0.05. The animals which were reared in greater herd size having 50 animals are at 81.81% more risk of having BoVH-1 antibodies followed by 64.06% and 62.5% by 20-50 animals and <20 animals respectively. Similarly, the owners which were using Artificial insemination services for breeding purpose to animals were at more threat 78.22% followed by 58.73% by natural/bull services. Rearing or housing pattern of animals was found a non-significant factor in rise of BoVH-1 antibodies titer in herd animals showing 66.66% and 69.59% sero-positivity in extensive and confined system, respectively (Table I).

Based on Univariate analysis, the potential risk factors were examined through odd ratio (OR>1). Bovine specie was found to be a non-significant risk factor (OR=0.55) (CI=0.31-0.95) for BoHV-1 sero-epidemiology. The Age although significantly associated on Chi-Square basis (\( P<0.05 \)) was not a potential risk factor upon univariate analysis OR=0.46(CI=0.24-0.88). Animal history proved to be a potential risk factor OR=10.86 (5.73-20.55) showing effects on disease dynamics. Another factor associated with BoHV-1 was herd size OR=2.52 (CI 1.22-5.19). Parity was also found positively associated with sero-prevalence of BoHV-1based on odd ratio OR=1.2 (CI=0.52-2.75) (Table I).

### Table I. Prevalence of BoHV-1 in cattle and buffalo, and associated risk factors in district Sheikhupura.

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>Positive (%)</th>
<th>( p ) value</th>
<th>Odd ratio</th>
<th>Confidence interval (95%) lower limit</th>
<th>upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>90</td>
<td>54 (60)</td>
<td>0.032</td>
<td>0.55</td>
<td>0.31-0.95</td>
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<tr>
<td>Buffalo</td>
<td>160</td>
<td>117 (73)</td>
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<tr>
<td>Age</td>
<td></td>
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<tr>
<td>&lt; 3 years</td>
<td>48</td>
<td>26 (54)</td>
<td>0.018</td>
<td>0.46</td>
<td>0.24-0.88</td>
<td></td>
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<tr>
<td>&gt; 3 years</td>
<td>202</td>
<td>145 (72)</td>
<td></td>
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<tr>
<td>Herd size</td>
<td></td>
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<tr>
<td>&lt; 20</td>
<td>56</td>
<td>35 (62.5)</td>
<td>0.018</td>
<td>0.935</td>
<td>0.48-1.79</td>
<td>2.52</td>
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<tr>
<td>&gt;= 20</td>
<td>128</td>
<td>82 (64)</td>
<td></td>
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<td></td>
<td>1.22-5.1</td>
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<td>Breeding method</td>
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<tr>
<td>Natural</td>
<td>126</td>
<td>74 (58.7)</td>
<td>0.001</td>
<td>0.39</td>
<td>0.22-0.68</td>
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</tr>
<tr>
<td>AI</td>
<td>124</td>
<td>97 (78.22)</td>
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<td>Management system</td>
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<tr>
<td>Extensive</td>
<td>02</td>
<td>68 (66.6)</td>
<td>0.625</td>
<td>0.87</td>
<td>0.50-1.5</td>
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<tr>
<td>Confined</td>
<td>148</td>
<td>103 (69.5)</td>
<td></td>
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<tr>
<td>History of animals</td>
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<tr>
<td>Abortion</td>
<td>145</td>
<td>128 (88.2)</td>
<td>0.000</td>
<td>10.86</td>
<td>5.73-20.55</td>
<td></td>
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<tr>
<td>Abortion + respiratory</td>
<td>105</td>
<td>43 (42.1)</td>
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<tr>
<td>Parity of animals</td>
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<tr>
<td>0-2</td>
<td>61</td>
<td>32(52.4)</td>
<td>0.008</td>
<td>0.41</td>
<td>0.22-0.76</td>
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<tr>
<td>3-4</td>
<td>151</td>
<td>110(72.8)</td>
<td>1.20</td>
<td>0.52-2.75</td>
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<tr>
<td>5-6</td>
<td>38</td>
<td>29(76.3)</td>
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</table>

Discussion

This study was designed for 250 samples of cattle and buffalo from densely populated areas of Sheikhupura district which is a first study on BoHV-1 and its associated risk factors in this district. Competitive ELISA was used to detect antibodies of BoHV-1.

Sero logical results depict, in 171 samples of dairy animals antibodies were circulating to show 68.5% sero-
positivity in targeted area. Results of this study are in close to prevalence noted previously in Lahore district (69.8%) (Shabbir et al., 2013). Our findings are also in accordance of results of another study conducted in India with 68.9% (Nandi et al., 2011) and 64.5% observed in various northern regions of Mexico (Erfani et al., 2019).

Our results indicate that sero-prevalence of buffalo is more than cattle 73.12% and 60%, respectively reflecting specie of animal a potent risk factor of BoHV-1 infection. Inflated BoHV-1 antibodies titer in countries with marked buffalo population and cattle are also be raised. Sero-survey have shown BoHV-1 infection have maintenance of 70.3% prevalence in buffalo (Shabbir et al., 2013). Proportion of buffalo sero-positivity in Italy was reported higher than cattle (Scicluna et al., 2007).

Data analysis of age as a risk factor unraveled high rise in prevalence of animals with more than 3 years of age (71.78%) in comparison to animals of age less than 3 years (54.16%). The earlier studies conducted in Sudan reveals that cattle of Sudan with age above 3 years were more susceptible (Elhassan et al., 2011). Other studies are also representing a rise in BoHV-1 infection with increasing age of animal (Derrar et al., 2019).

After evaluating data, in this study herd size is revealed as a significant risk factor along with age of animal. Similarly, a significant association was shown of herd size and BoHV-1 infection in statistical analysis (Dias et al., 2019). Another study was conducted in Lithuania revealed a relation of herd size with BoHV-1 infection (Jacevičius et al., 2010). In this study, animals bred with artificial insemination have been shown a rise in sero-positivity of BoHV-1 rather to animals bred with service bull 78.73% and 58.73% respectively. Insemination of contaminated semen through AI tends to intermittent estrus, fall in conception rate and endometritis with BoHV-1 (Nandi et al., 2009).

Animals kept in intensive housing system were at greater risk of disease than non-tethered herds. Previous study showed that housing management had a direct proportion with virus prevalence (Chandranai et al., 2014). Our study has recorded a poor correlation of housing management with BoHV-1 infection. Animals kept in extensive or confined management have been 66.66 % and 69.59% sero-prevalence, respectively.

Data examination illustrated high sero-prevalence in multi-parous animals in comparison to nulliparous animals. A study in India showed that IBR antibodies titer surged as parity of animal increased. Scientists investigated IBR antibodies up to 4th lactation and found that highest antibody titer was noted in animals with 4th lactation (Kathiriya et al., 2018). Cows with parturition history of 6 or more had highest sero-positivity 82.4% (Romero-Salas et al., 2018).

Statement of conflict of interest
The authors have declared no conflict of interest.

References
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