Molecular Detection and Epidemiology of Equine Herpesvirus 2 and 5 in Working Equids in Central Ethiopia

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Research Article

Keywords: EHV-2, EHV-5, Equids, PCR, Respiratory disease, Ethiopia

Posted Date: January 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-130965/v1

Abstract

Background: Equine respiratory illness is a common problem that impacts the performance of the working capacity of equids. In Ethiopia, respiratory disease is the most common presenting complaint at veterinary clinics and a priority concern for equid owners and veterinary practitioners. Although studies had been conducted in EHV-2 and EHV-5 elsewhere in the world, many unknowns remained. Thus, a detailed study is needed to understand more about the epidemiology of the viruses. This study aimed to detect EHV-2 and EHV-5 from working equids in central Ethiopia.

Methods: Nasopharyngeal swabs were collected from 58 horses and donkeys to detect EHV-2 and EHV-5 using PCR. Viral DNA was extracted and PCR amplification was performed using virus-specific primers targeting the conserved region of glycoprotein B (gB) genes.

Results: From 58 equids, EHV-5 and EHV-2 were detected in 20 (34.5%) and 19 (32.8%) equids, respectively. Concurrent infection with EHV-2 and EHV-5 was found in 6 (10.3%) diseased equids. EHV-2 was detected in a significantly higher proportion (P = 0.002) in horses (54.5%; n = 18) than donkeys (4%; n = 1). In contrast, a significantly higher (P = 0.004) proportion of EHV-5 was detected in donkeys (56%; n =14) than horses (18.2%; n = 6). EHV-2 was detected in a significantly higher (P = 0.006) proportion in equids displaying signs of respiratory disease (16/33; 48.5%) compared to those without disease (3/25; 12%). EHV-2 positive equids were seven times more likely to display clinical signs of respiratory disease than EHV-2-negative equids (OR = 6.9; 95% CI: 1.72-27.60). For EHV-5, the observed difference was not statistically significant (P = 0.832). A significantly higher (P = 0.041) proportion of EHV-2 was detected in equids living in midland (52.9%; n = 9) compared with highland (24.4%; n = 10). Equids residing in the midland were four times more likely to be exposed to EHV-2 than highland equids (OR = 3.97; 95% CI: 1.05 - 14.89).

Conclusion: EHV-2 and EHV-5 are highly prevalent both in horses and donkeys residing in central Ethiopia. Species-specific susceptibility differences in EHV-2 and EHV-5 infection are observed. The observed causal association between EHV-2-test-positive and the appearance of clinical signs of respiratory disorders should be further investigated.

Introduction

Approximately 60% of the world's horse population and over 95% of all donkeys and mules are found in developing countries [1]. According to the survey carried out by the Ethiopian Central Statistical Agency (CSA), there are about 8.85 million donkeys, 2.01 million horses, and 0.46 million mules recorded in Ethiopia [2], probably with the highest density per square kilometer in the world. Equids have an essential role in the livelihoods of millions of people in Ethiopia mainly used for transportation, agricultural purposes, and other social values. Notwithstanding basic husbandry and welfare needs, infectious diseases compromise the health and welfare of working equids in Ethiopia. Among the multiple

infectious disease problems affecting working equids, equine herpesviruses are one of the major viral respiratory pathogens that threaten the health of equids [3].

Equine herpesviruses (EHVs) are enveloped DNA viruses that have a major economic and welfare impact on the horse industry. Equine herpesvirus 2 (EHV-2) and equine herpesvirus 5 (EHV-5) are classified within the genus *Percavirus* of the family of *Gammaherpesvirinae*, which are recognized as infecting horses as their natural host [4, 5]. Infections caused by EHV-2 and EHV-5 usually occur during the early stages of the life of horses. These viruses can establish lifelong latency in a large proportion of equids that ensures their survival in equine populations and enables the virus to be shed sporadically throughout the lifetime of the host [6]. Horses with latent and/or active infection harbor these viruses and serve as carriers and reservoirs of infections. These carriers become disseminators of the viruses when they are stressed (transported, raced, starved, etc.) [7] and immune-compromised [8]. EHV-2 infection usually associated with keratoconjunctivitis, upper respiratory disease, pneumonia, chronic follicular pharyngitis, and fever [9, 10] whereas EHV-5 typically causes upper respiratory tract disease (e.g. pharyngitis) or keratoconjunctivitis accompanied by clinical signs such as nasal and ocular discharge, tachypnea, coughing, fever, enlarged lymph nodes, anorexia, poor body condition, and depression [11, 12]. EHV-5 has also been implicated as an etiologic agent for fatal equine multinodular pulmonary fibrosis [13, 14].

Respiratory pathogens are important causes of disease in equine populations worldwide. Previous studies conducted in Ethiopia have documented that respiratory problem particularly coughing and nasal discharge is one of the major health concerns for working equids in Ethiopia [15]. Equine herpesviruses are important pathogens that are involved in respiratory problems of varying severity [16, 17]. Epidemiological studies have reported that the severity of the diseases associated with EHV-2 and EHV-5 is often influenced by various risk factors such as the age, physical condition, immune status, and breed of the host [18, 19]. However, little is known about the disease burden and their epidemiology in Ethiopian working equids. The knowledge on the epidemiology of equine gammaherpesviruses is important to a better understanding of the impact of the disease on the well-being of the working equids. Thus, the aim of this study was molecular detection of EHV-2 and EHV-5 and to investigate the factors associated with infection among working equids in central Ethiopia.

Results

Virus-specific PCR were used to detect EHV-2 and EHV-5 from 58 nasopharigial swabs collected from horses and donkeys. From a total of 58 equids, 19 (32.8%) and 20 (34.5%) equids were found positive for EHV-2 (Table 2 and Fig. 2) and EHV-5 (Table 3 and Fig. 3), respectively (Tables 2 and 3). EHV-2 was detected in a significantly higher proportion (P = 0.002) in horses (54.5%; n = 18) than donkeys (4%; n = 1) (Table 2). In contrast, a significantly higher (P = 0.004) proportion of EHV-5 was detected in donkeys (56%; n = 14) compared with horses (18.2%; n = 6) (Table 3).

The proportion of positive equids for EHV-2 and EHV-5 was varied between males and femals. A significantly higher (P = 0.004) proportion of EHV-2 was detected in male (50%; n = 16) compared to

female equids (11.5%; n = 3) (Table 2). In contrast, although not statistically significantly (P = 0.566), EHV-5 was detected in a higher proportion in female (38.4%; n = 10) than male equids (31.3%; n = 10) (Table 3).

The presence of EHV-2 and EHV-5 was varied with different age groups as shown in Tables 2 and 3. A higher proportion of EHV-2 was detected in yearlings and young equids (42.9%; n = 6) compared with adults (34.3%; n = 12) and old ages (11.1%; n = 1), however, the observed difference was not statistically significantly (P > 0.05). In contrast, although not significant (P > 0.05), EHV-5 was detected in a higher proportion in older equids (55.6%; n = 5) compared with adults (31.4%; n = 11) and young equids (28.6%; n = 4).

The proportion of EHV-2 and EHV-5 was compared between equids displaying clinical signs of respiratory disease (n = 33) and equids without clinical signs of illness (n = 25). Accordingly, a significantly higher (P = 0.006) proportion of EHV-2 was observed in equids with signs of respiratory disease (16/33; 48.5%) compared to those without the disease (3/25; 12%). The odds of being positive for EHV-2 in equids displaying clinical signs of respiratory disease was nearly 7 times higher than equids without showing clinical signs (OR = 6.9; 95% CI: 1.72–27.60) (Table 2). In contrast, EHV-5 was detected in a slightly, but not significantly (P = 0.832), higher proportion in apparently healthy (36%; n = 9) equids compared to equids displaying clinical signs of respiratory disease (33.3%; n = 11) (Table 3). Concurrent infection with EHV-2 and EHV-5 was found in 6 (10.3%) equids exhibiting respiratory clinical signs.

The proportion of equids infected with EHV-2 and EHV-5 was varied within the different geographical locations of the sampling sites. A significantly higher (P = 0.041) proportion of EHV-2 was detected in equids living in midland (52.9%; n = 9) compared with highland (24.4%; n = 10). Equids residing in the midland were four times more likely to be exposed to EHV-2 than highland equids (OR = 3.97; 95% CI: 1.05–14.89) (Table 2). In contrast, a higher proportion of EHV-5 was found in equids residing in highlands (39.0%; n = 16) compared with equids living in midland (23.5%; n = 4), however, this difference was not statistically significant (P = 0.444) (Table 3).

Discussion

Equine respiratory infection is the most common problem that impacts the performance of the working ability of equids. In Ethiopia, respiratory disease (coughing and nasal discharge) is the most common presenting complaint at veterinary clinics and a priority concern for owners of the working equids and veterinary practitioners. Equine herpesviruses are important pathogens that are involved in respiratory problems of varying severity. In the present study, the detection of equine gammaherpesviruses (EHV-2 and EHV-5) from horses and donkeys in central Ethiopia is described. We also assessed the risk factors associated with respiratory disease and EHV-2/5 clinical outcomes.

In the present study, from a total of 58 equids, 34.5% and 32.8% were tested positive for EHV-5 and EHV-2, respectively. This shows that both EHV-2 and EHV-5 are prevalent in horses and donkeys residing in central Ethiopia. In this study, EHV-5 was recorded relatively in a higher proportion than EHV-2. This finding is consistent with previous studies conducted by Diallo et al. [20] and Wang et al. [21] in Australia

and Negussie et al. [16] in Ethiopia, but in contrast to Dunowska et al. [11] in New Zealand, Nordengrahn et al. (2002) [23] in Sweden, Hungary, and UK and Torfason et al. [24] in Iceland in which EHV-2 has been recorded in a higher prevalence than EHV-5.

In this study, EHV-2 was detected in a significantly higher proportion of horses (54.5%) than donkeys (4%). In contrast, a significantly higher proportion of EHV-5 was detected in donkeys (56%) as compared with horses (18.2%). Our result demonstrated the presence of gammaherpesviruses susceptibility variation between host species. The existence of breed-specific susceptibility differences [19] and species-specific susceptibility differences [16] has been reported. However, for more validation, this variation in susceptibility should be further investigated using large sample size.

Co-infection of EHV-2 and EHV-5 was found in 6 (10.3%) equids displaying clinical signs of respiratory disease. This dual infection is consistent with other reports [9, 16, 23, 25] in which both viruses can simultaneously infect equids. This co-infection may have a synergistic effect on disease outcome and may lead to severe respiratory disease, however, need further investigation.

EHV-2 and EHV-5 were found test positive from 16 (48.5%) and 11 (33.3%) equids exhibiting clinical signs of respiratory disease, respectively. Similarly, EHV-2 and EHV-5 were tested positive from 3 (12%) and 9 (36%) equids with no signs of illness, respectively. The existence of EHV-2 and EHV-5 in apparently healthy equids is in agreement with previous studies conducted elsewhere [16, 19, 25, 26]. A significantly higher proportion of EHV-2 was detected in equids with signs of respiratory disease (48.5%) as compared to those without clinical signs (12%). EHV-2 positive equids were seven times more likely to display clinical signs of respiratory disease than EHV-2-negative equids (OR = 6.9; 95% CI: 1.72–27.60). This causal association between EHV-2-test-positive and the appearance of clinical signs of respiratory disorders may suggest the involvement of EHV-2 in the development of respiratory diseases. Negussie et al. [16] proposed that EHV-2 may have a possible etiological contribution either to induce or predispose equids to respiratory diseases. Ataseven et al. [25] stated that EHV-2 played a pre-disposing role in the occurrence of respiratory diseases. However, the presence of a statistical association between EHV-2-test-positive and displaying of clinical signs alone is not sufficient to causal linkage with the disease, thus, further detailed studies on the pathogenesis are necessary to unequivocally conclude a causal association between EHV-2 and induction of clinical symptoms.

In this study, a higher proportion of EHV-2 was detected in yearlings and young equids (42.9%; n = 6) as compared with adults (34.3%; n = 12) and old ages (11.1%; n = 1), however, the observed difference was not statistically significantly ($P > 0.05$). Our study is in agreement with previous studies where equids are highly infected with EHV-2 in the first years of life despite the presence of maternal antibodies in the colostrum [12, 23, 27]. Similarly, although not significant ($P > 0.05$), EHV-5 was detected in a higher proportion in older equids (55.6%; n = 5) as compared with adults (31.4%; n = 11) and young (28.6%; n = 4) (Table 3). Some epidemiologic evidence suggests that foals are usually infected with EHV-5 later on in life than with EHV-2 [23, 27].

The proportion of equids infected with EHV-2 and EHV-5 was varied in different geographical locations of the sampling sites. A significantly higher (P = 0.041) proportion of EHV-2 test positive was found in equids residing in midlands (52.9%) as compared with highlands (24.4%). Equids residing in the midlands were four times more likely to be exposed to EHV-2 than highlands equids (OR = 3.97; 95% CI: 1.05–14.89). In contrast, a higher proportion of EHV-5 was found in equids residing in highlands (39.0%) as compared with midlands (23.5%), however, this difference was not statistically significant (P = 0.444). This study suggests a wide distribution and varying susceptibility to the viruses in different agro-ecology. Azab et al. [28] reported that the variation in the prevalence of the disease among studies is attributed to geographical variability and environmental factors. However, at present, a possible explanation of why equids residing in different agroecology have varying susceptibility to EHV infection could not be given.

In conclusion, EHV-2 and EHV-5 are highly prevalent both in horses and donkeys residing in central Ethiopia. Concurrent infection with EHV-2 and EHV-5 was recorded. Species-specific susceptibility differences in EHV-2 and EHV-5 infection are observed. Our result suggests the involvement of EHV-2 in the development of respiratory diseases. However, further studies are necessary to unequivocally conclude respiratory illness associated with EHV-2.

Methods

Study areas

This study was conducted from November 2019 to February 2020 in selected districts of the central part of Ethiopia such as AngolelanaTera (Chacha), Basona Werana (Debre Berhan Zuria), Kembebit (Sheno), Bishoftu (Ade'a), and Arsi Negele districts (Fig. 1). These districts were selected based on previous respiratory disease reports and the presence of high number of equine population.

Samples were collected from six districts of different altitudes and locations of the sampling sites were recorded by a global positioning system (GPS). The study areas consist of two agroecological zones: highlands (above 2500 m above sea level (masl) and midland (between 1500–2500 masl). The study was conducted in the highlands of Angolelana Tera (Chacha) of Amhara region with an altitude of 2812 masl and in Kembibit of Oromia region with an altitude ranges from 2630–3020 masl. The study was also conducted in the midlands of Basona Werena (Debre Berhan zuria) of Amhara region with an altitude of 2360 masl and Ada'a (Bishoftu), and Arsi Negele districts of Oromia region which are located at an altitude of 1900 masl and an altitude ranges from 1500 to 2300 masl, respectively.

Sample collection

In each study site, equids were clinically examined for evidence of respiratory distress, coughing, and nasal discharge. Equids without noticeable respiratory clinical signs were also included in this study. Epidemiological data such as age, sex, species of the host, presence of clinical signs, and geographical locations were also collected to determine whether viral detection was associated with potential risk factors. A total of 58 equids composed of 25 donkeys and 33 horses were enrolled in this study. The

study population was also categorized into three age groups: ≤3 years (yearlings and young equids), 4–10 years (adults), and above 10 years (old).

Nasopharyngeal swabs were collected from equids with clinical signs of respiratory disorder and from those equids without noticeable clinical signs. Nasopharyngeal swabs were collected using standard Sigma Virocult® swab – 15 cm long with a cellular foam bud. Each collected swabs was placed into three ml viral transport medium containing phosphate-buffered saline (PBS) with a pH of 7.2–7.6 and antibiotics. Epidemiological information and disease conditions were also recorded during sampling. All collected samples were labeled and immediately placed in a cooler containing ice and transported to the National Veterinary Institute, Bishoftu. Samples were kept at – 20 °C until further processing.

DNA Extraction and Polymerase Chain Reaction (PCR) Assay

Viral DNA extraction was performed from 200µl of nasopharyngeal swabs using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was stored at -20^{0}C until processed. PCR amplification was performed using virus-specific primers targeting the conserved region of glycoprotein B (gB) genes for EHV-2 (444 bp) and EHV-5 (293 bp) according to [20] and [29], respectively (Table 1).

Each PCR was performed using Agilent's Herculase II fusion DNA polymerase (Agilent Technologies, Inc., Santa Clara, CA, USA). Each reaction was processed in a total volume of 25 µL mixture containing 12.5 µl of nuclease-free water, 5 µl of Herculase reaction buffer, 0.5 µl Herculase II fusion DNA polymerase, 0.5 µl of 25 mM each deoxynucleoside triphosphate (dNTP) mix, 1 µl of each forward and reverse primers, 2.5 µl of dimethyl sulphoxide (DMSO), and 2 µl template DNA. In each reaction, nuclease-free water and known EHV-2 and EHV-5 DNA extract were used as a negative and positive control, respectively.

PCR assay targeting the region of gB genes of EHV-2 and EHV-5 was amplified using the following thermocycling conditions: an initial denaturation step of 95°C for 5 min, followed by 40 cycles of amplification, using denaturation at 95°C for 30 s, annealing at 60°C and extension at 72°C for 45 s and followed by a final extension at 72°C for 10 minutes. The final PCR products were visualized using 1.5% agarose gel electrophoresis using electrolyte buffer Tris Acetate EDTA (TAE) and stained with gel red under UV light. The gels were examined for specific size bands using a UV transilluminator.

Statistical Analysis

Data generated from laboratory investigations were recorded using Microsoft Excel spreadsheets and analyzed using STATA version 14 for Windows (Stata Corp. College Station, TX, USA). Logistic regression analysis was employed to investigate associations between risk factors and EHV-2/5 detection. Differences were considered as statistically significant when *P*-value was < 0.05.

Abbreviations

bp: base pair

DMSO: dimethyl sulphoxide

DNA: Deoxyribonucleic acid

dNTP: deoxynucleoside triphosphate

EHV: Equine herpesvirus

gp: glycoprotein

Masl: Meter above sea level

OR: Odds ratio

PCR: Polymerase chain reaction

UV: Ultraviolet

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

The authors would like to acknowledge the National Veterinary Institute and the National Animal Health Diagnostic and Investigation Center, Ethiopia, for the provision of laboratory facilities. We also acknowledge the equine owners for their willingness and committed to support the success of this research work.

Funding

This study was supported by the Addis Ababa University thematic research fund (VPRTT/PY-096/2018), Ethiopia. The funder had no role in the conception, design of the study, data collection, analysis and interpretation of the data reported in this manuscript.

Authors' contributions

KW performed field sample collection, laboratory analysis, and wrote the draft manuscript. SL participated in data analysis. KA analyzed the data and participated in reviewing the manuscript. HN set

up the study designs, participated in sample collection and laboratory analysis, coordinated the work, and wrote the manuscript. All authors read and approved the final manuscript.

Ethics declarations

Ethical approval and consent to participate

Ethical approval for this study was granted from the animal research ethical review committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University (Reference number: VM/ERC/08/01/12/2020). All methods were performed in accordance with relevant guidelines and regulations. All protocols were approved by animal research ethical review committee. Before conducting the research, equine owners were informed with the objectives and the benefits of the study and they gave consent for their animal's inclusion in the study. The informed consent was obtained from all participating equine owners prior to sample collection and this was approved by the ethics committee. The consent from equine owners was verbal because they are unable to write and read. These consents were taken in the presence of a third independent party.

Consent for publication

Consent to publish the finding of the data was obtained verbally from all equine owners during sampling and data collection.

Competing interests

The authors declare that they have no competing interests.

References

1. Pritchard J, Lindberg A, Main D, Whay H. Assessment of the welfare of working horses, mules and donkeys, using health and behaviour parameters. Prev Vet Med. 2005;69:265–83.
2. CSA. Report on livestock and livestock characteristics (private peasant holdings). Addis Ababa, Ethiopia; 2018.
3. Getachew, M. Alemayehu, F. Chala, C. Amare, B. Kassa, D. Burden, F. Wernery, R. Wernery U. A cross-sectional sero-survey of some infectious diseases of working equids in Central Ethiopia. J Vet Med Anim Heal. 2014;6:231–8.
4. Davison AJ, Eberle, R., Ehlers B, Hayward GS, McGeoch DJ, Minson AC, Pellett PE, et al. The Order Herpesvirales. Arch Virol. 2009;154:171–7.
5. King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz EJSD. Virus taxonomy: classification and nomenclature of viruses. In: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press; 2012.
6. Gilkerson JR, Bailey KE, Diaz-Méndez A, Hartley CA. Update on Viral Diseases of the Equine Respiratory Tract. Vet Clin North Am Equine Pract. 2015;31:91–104.

7. Muscat KE, Padalino B, Hartley CA, Ficorilli N, Celi P, Knight P, Raidal S, Gilkerson JR. Equine Transport and Changes in Equid Herpesvirus ' Status. Front Vet Sci. 2018;5 September:1–10.
8. Ma G, Azab W, Osterrieder N. Equine herpesviruses type 1 (EHV-1) and 4 (EHV-4)-Masters of co-evolution and a constant threat to equids and beyond. Vet Microbiol. 2013;167:123–34.
9. Allen G, Murray M. Equid herpesvirus 2 and equid herpesvirus 5 infections. In: Infectious Diseases of Livestock, (Ed.) JAW Coetzer and RC Tustin. Oxford Press (Cape Town). 2004. p. 860–7. http://www.ca.uky.edu/gluck/images/faculty pages/Equid herpesvirus 2 and equid herpesvirus 5 infections.pdf.
10. Fortier G, van Erck E, Pronost S, Lekeux P, Thiry E. Equine gammaherpesviruses: Pathogenesis, epidemiology and diagnosis. Vet J. 2010;186:148–56.
11. Dunowska M, Wilks CR, Studdert MJ, Meers J. Equine respiratory viruses in foals in New Zealand. N Z Vet J. 2002;50:140–7.
12. Rushton JO, Kolodziejek J, Tichy A, Nell B, Nowotny N. Detection of equid herpesviruses 2 and 5 in a herd of 266 Lipizzaners in association with ocular findings. Vet Microbiol. 2013;164:139–44.
13. Dunowska M, Hardcastle MR, Tonkin FB. Identification of the first New Zealand case of equine multinodular pulmonary fibrosis. N Z Vet J. 2014;62:226–31.
14. Williams, K. J., R. Maes, F. Del Piero, A. Lim, A. Wise, D. Bolin, J. Caswell, C. Jackson, N. Robinson and FD. Equine multinodular pulmonary fibrosis: a newly recognized herpesvirus-associated fibrotic lung disease. Vet Pathol. 2007;44:849–862.
15. Laing, G. Christley, R. Stringer, A. Aklilu, N. Ashine, T. Newton, R. Radford, A. Pinchbeck G. Respiratory disease and sero-epidemiology of respiratory pathogens in the working horses of Ethiopia. Equine Vet J. 2018;50:793–9.
16. Negussie H, Gizaw D, Tesfaw L, Li Y, Oguma K, Sentsui H, Tessema TS NH. Detection of Equine Herpesvirus (EHV) -1, -2, -4 and -5 in Ethiopian Equids with and without Respiratory Problems and Genetic Characterization of EHV-2 and EHV-5 Strains. Transbound Emerg Dis. 2017;64:1970–8.
17. Negussie H, Gizaw D, Tessema TS, Nauwynck HJ. Equine Herpesvirus-1 Myeloencephalopathy , an Emerging Threat of Working Equids in Ethiopia. Transbound Emerg Dis. 2015;:1–9.
18. Agnol, A. D., Beuttemmüller, E. A., Pilz, D., Oliveira, M. V., Headley, S. A., Ferraz, L. E. S., … Alfieri AA. Detection of equine herpesvirus-2 and -5 infection in asymptomatic horses from Brazil. J Equine Vet Sci. 2016;39:3220.
19. Stasiak K, Dunowska M, Rola J. Prevalence and sequence analysis of equid herpesviruses from the respiratory tract of Polish horses. Virol J. 2018;15:1–13.
20. Diallo, I.S., G.R. Hewitson, A. Jong, M.A. Kelly, D.J. Wright, B.G. Corney, and B.J. Rodwell. Equine herpesvirus infections in yearlings in South-East Queensland. Arch Virol. 2008;153:1643–1649.
21. Wang L, Raidal SL, Pizzirani A, Wilcox GE. Detection of respiratory herpesviruses in foals and adult horses determined by nested multiplex PCR. Vet Microbiol. 2007;121:18–28.

22. Nordengrahn A, Merza M RC, Nordengrahn A, Merza M, Ros C et al. Prevalence of equine herpesvirus types 2 and 5 in horse populations by using type-specific PCR assays. Vet Res. 2002;33:251–259.
23. Nordengrahn A, Merza M, Ros C, Lindholmc A, Palfl V, Hannant D, et al. Prevalence of equine herpesvirus types 2 and 5 in horse populations by using type-specific PCR assays. Vet Res. 2002;33:251–259.
24. Torfason EG, Thorsteinsdottir L, Torsteinsdóttir S S V. Study of equid herpesviruses 2 and 5 in Iceland with a type-specifc polymerase chain reaction. Res Vet Sci. 2008;85:605–611.
25. Ataseven VS, Bilge-Dagalp S, Oguzoglu TC, Karapinar Z, Guzel M, Tan MT. Detection and sequence analysis of equine gammaherpesviruses from horses with respiratory tract disease in Turkey. Transbound Emerg Dis. 2010;57:271–6.
26. Laabassi F, Hue E, Fortier C, Morilland E, Legrand L, Hans A PS. Epidemiology and molecular detection of equine herpesviruses in western Algeria in 2011. Vet Microbiol. 2017;207 January:205–9.
27. Bell SA, Balasuriya UBR, Gardner IA, Barry PA, Wilson WD, Ferraro GL, et al. Temporal detection of equine herpesvirus infections of a cohort of mares and their foals. Vet Microbiol. 2006;116:249–57.
28. Azab W, Bedair S, Abdelgawad A, Eschke K, Farag GK, Abdel-Raheim A, Greenwood AD, Osterrieder N AA. Detection of equid herpesviruses among different Arabian horse populations in Egypt. Vet Med Sci. 2019;5:361–71.
29. Holloway SA, Lindquester GJ, Studdert MJ, Drummer HE. Identification, sequence analysis and characterisation of equine herpesvirus 5 glycoprotein B. Arch Virol. 1999;144:287–307.

Tables

Table 1

Primers used for amplification of a region of the gB genes of EHV-2 and EHV-5

Virus	Region	PCR Primers	Size	Reference
EHV-2	gB	FW: 5′GCCAGTGTCTGCCAAGTTGATA-3′ RV: 5′-CATGGTCTCGATGTCAAACACG-3′	444 bp	[20]
EHV-5	gB	FW: 5′-ATGAACCTGACAGATGTGCC-3′ RV: 5′-CACGTTCACTATCACGTCGC-3′	293 bp	[29]

Table 2

Multivariable logistic regression analysis of risk factors associated with EHV-2 detection

Variables	No. of equids	EHV-2 positive (%)	OR	95% Confidence Interval	*P*-value
Species					
Donkey	25	1 (4.0%)	0.035	0.05 - 0.29	0.002
Horse	33	18 (54.5%)	1.0		
Sex					
Female	26	3 (11.5%)	1.0		
Male	32	16 (50.0%)	7.67	1.91 - 30.73	0.004
Age group					
≤3	14	6 (42.9%)	1.44	0.40 - 5.11	0.575
4-10	35	12 (34.3%)	1.0		
>10	9	1 (11.1%)	0.24	0.03 - 2.14	0.202
Location					
Midlands	17	9 (52.9%)	3.97	1.05 - 14.89	0.041
Highland	41	10 (24.4%)	1.0		
Respiratory disease					
Yes	33	16 (48.5%)	6.90	1.72-27.60	0.006
No	25	3 (12.0%)	1.0		
Total	58	19(32.8%)			

Table 3

Multivariable logistic regression analysis of risk factors associated with EHV-5 detection

Variables	No. of equids	EHV-5 positive (%)	OR	95% Confidence interval	*P*-value
Species					
Donkey	25	14 (56.0%)	5.73	1.75 - 18.75	0.004
Horse	33	6 (18.2%)	1.0		
Sex					
Female	26	10 (38.4%)	1.0		
Male	32	10 (31.3%)	0.73	0.25 - 2.16	0.566
Age group					
≤3	14	4 (28.6%)	0.87	0.22 - 3.41	0.845
4-10	35	11 (31.4)	1.0		
>10	9	5 (55.6%)	2.73	0.61 - 12.17	0.189
Location					
Midlands	17	4 (23.5%)	0.5686	0.14 - 2.39	0.444
Highland	41	16 (39.0%)	1.0		
Respiratory disease					
Yes	33	11 (33.3%)	0.88	0.29 - 2.65	0.832
No	25	9 (36.0%)	1.0		
	58	20(34.5%)			

Figures

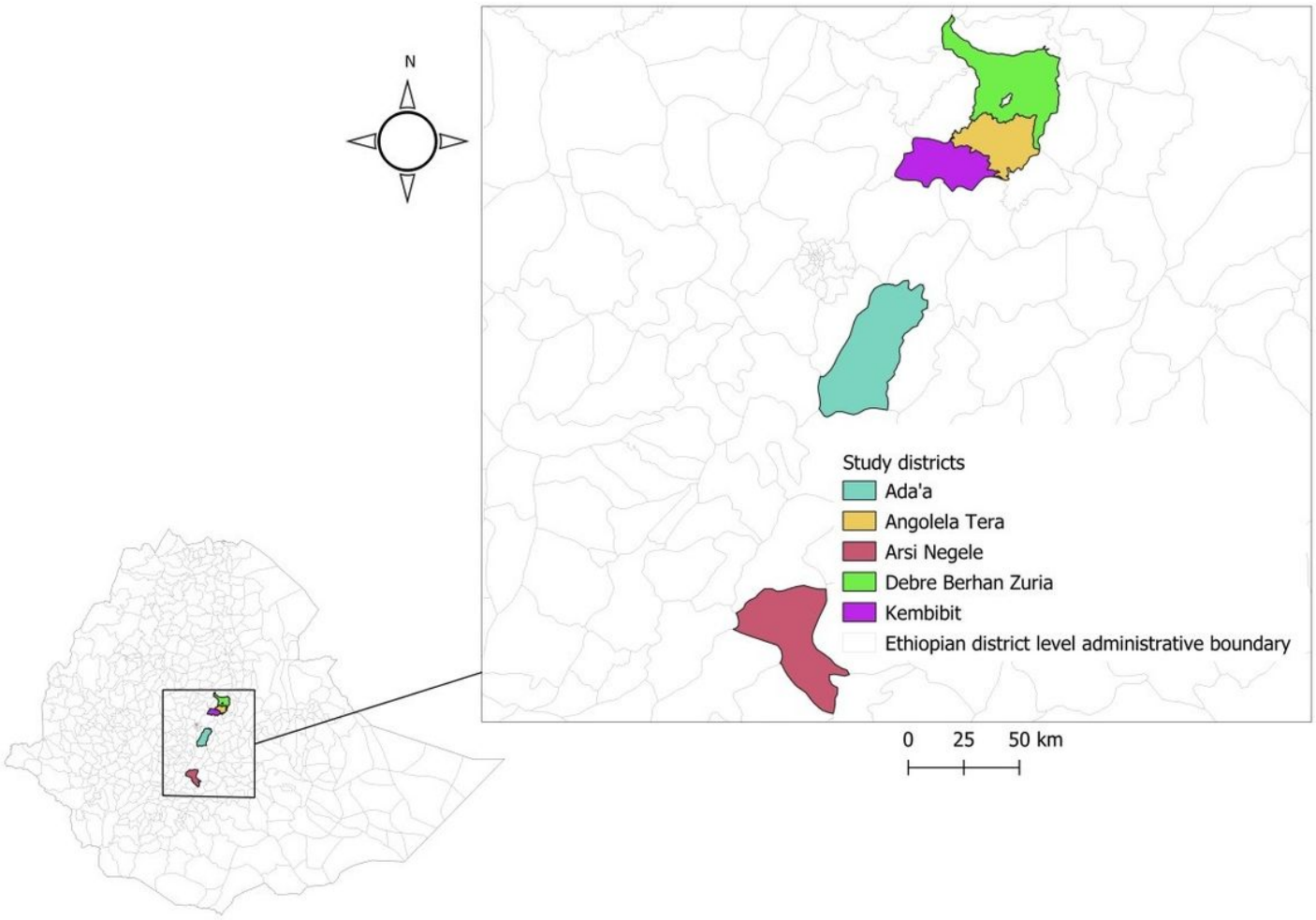

Figure 1

Map of Ethiopia showing the study sites: Add'a (Bishoftu), Angolelana Tera (Chacha), Aresi Negele, Debre Berhan zuria (Basona Werana), Kimbibit (Sheno). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

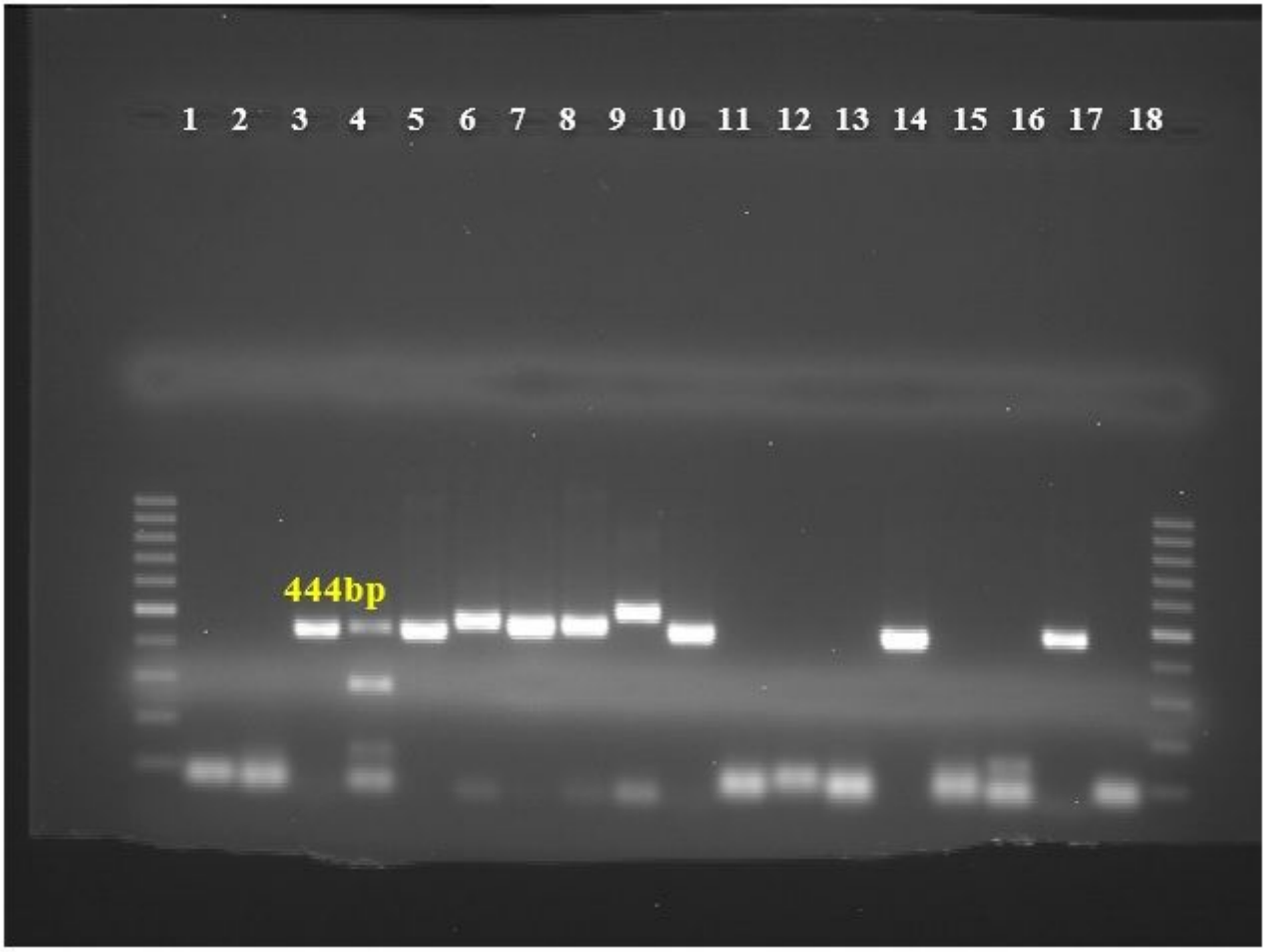

Figure 2

Gel document shows the specific regions of EHV-2 gpB gene (444bp) on 1.5% agaros gel with a DNA Molecular Weight Marker of 100bp; lanes 3 – 10 and 14 shows positive bands for EHV-2, lanes 1, 2, 11, 12, 13, 15 and 16 are negative samples, lane 17 and 18 are positive and negative control, respectively.

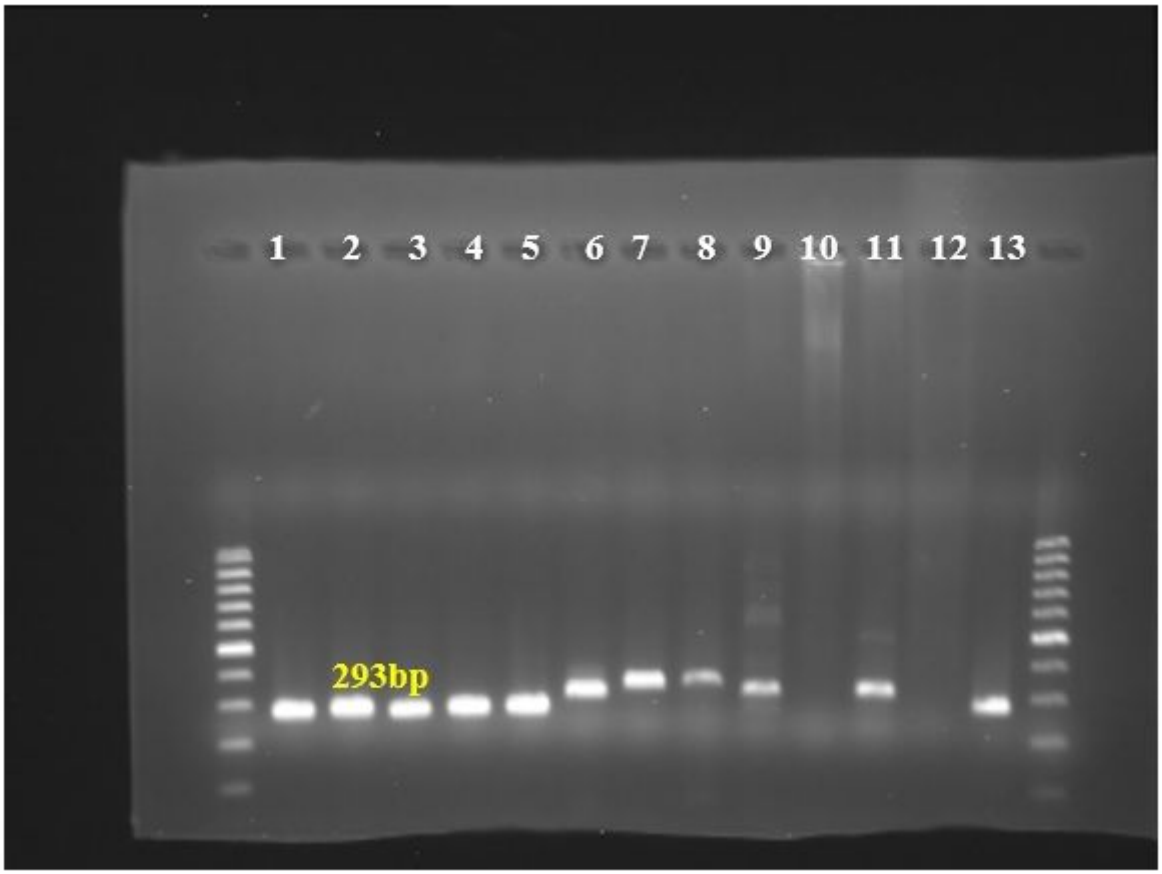

Figure 3

Gel document shows the specific regions of EHV-5 gpB gene (293bp) on 1.5% agarose gel with a DNA Molecular Weight Marker of 100bp; lanes 1 -9 and 11 show positive bands for EHV-5; lane 10 negative sample; lane 12 and 13 are negative and positive control, respectively.