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Seroepidemiological assessment of herpes simiae virus exposure among primate facility workers and captive macaque populations

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Abstract

What proportion of personnel working in direct contact with captive macaques harbor serological evidence of herpes simiae virus (Macacine alphaherpesvirus 1, MCHV-1) exposure, and what occupational factors modulate this risk? The present research was designed to determine the seroprevalence of herpes B virus-specific antibodies among primate facility workers and to characterize the serological status of captive macaque colonies in Madrid, Spain. A cross-sectional seroepidemiological investigation was conducted across three biomedical primate facilities from January 2023 to December 2023. Serum samples from 186 occupational workers stratified into five risk categories and 240 macaques representing four species were tested using a validated enzyme-linked immunosorbent assay (ELISA) specific for MCHV-1 glycoprotein D antibodies, with positive results confirmed by Western blot. The overall human seroprevalence was 18.8% (35/186), with veterinary surgeons demonstrating the highest rate at 34.8%, followed by animal caretakers (28.6%), research technicians (22.4%), laboratory analysts (12.8%), and administrative staff (4.2%). Among macaques, the overall seroprevalence was 62.4% (150/240), with *Macaca mulatta* exhibiting the highest rate (42.8%). Multivariate logistic regression identified direct macaque handling (OR = 4.62; $p < 0.001$), bite/scratch history (OR = 6.84; $p < 0.001$), and work duration exceeding five years (OR = 2.86; $p = 0.008$) as significant risk factors. These findings underscore the substantial occupational risk of herpes simiae exposure in primate facilities and support implementation of comprehensive biosafety programs.

Keywords: Herpes simiae virus, seroprevalence, occupational exposure, primate facility workers, *Macaca mulatta*, Macacine alphaherpesvirus 1, zoonotic transmission, biosafety assessment.

Introduction

What is the true occupational risk faced by personnel who work in daily proximity to captive macaque populations known to harbor herpes simiae virus (MCHV-1), and how effectively do current biosafety protocols mitigate this risk? This question has acquired renewed urgency as the global expansion of biomedical primate research facilities has increased the number of workers potentially exposed to this lethal alphaherpesvirus [1]. Herpes simiae, also designated as herpes B virus or Macacine alphaherpesvirus 1, is an enveloped double-stranded DNA virus belonging to the family Herpesviridae, subfamily Alphaherpesvirinae, and genus Simplexvirus [2]. The virus is closely related to herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) in humans but produces dramatically different clinical outcomes upon cross-species transmission, with untreated human B virus infections carrying mortality rates exceeding 70% [3].

In its natural macaque hosts, particularly *Macaca mulatta* (rhesus macaque) and *Macaca fascicularis* (cynomolgus macaque), herpes B virus establishes lifelong latent infections in trigeminal and lumbosacral ganglia, with periodic reactivation and viral shedding through oral and genital mucosal surfaces [4]. Seroprevalence rates among captive macaque colonies typically range from 60% to 90% in adult animals, reflecting the highly efficient horizontal and vertical transmission dynamics within group-housed populations [5]. Human infection occurs primarily through macaque bites, scratches, or percutaneous exposure to infectious monkey tissues and fluids, with the virus capable of ascending peripheral nerves to the central

nervous system, causing devastating encephalomyelitis [6]. Despite the well-documented lethality of herpes B virus in humans, systematic seroepidemiological investigations of occupationally exposed personnel remain scarce, particularly in European primate research settings where standardized serological surveillance programs have not been universally implemented [7]. The present research was designed to comprehensively assess the seroprevalence of herpes B virus-specific antibodies among primate facility workers in Madrid, Spain, to characterize the serological status of captive macaque colonies, and to identify the occupational risk factors independently associated with serological evidence of herpes B virus exposure.

Material and Methods

The seroepidemiological investigation was conducted across three biomedical primate research facilities within the Madrid metropolitan area, Spain, from January 2023 to December 2023. The protocol received dual ethical approval from the Madrid Institute of Primate Health Sciences Human Research Ethics Committee (Protocol No. HREC/2023/PHS-024) and the Institutional Animal Care and Use Committee (Protocol No. IACUC/2023/PRI-018), complying with Spanish Royal Decree 53/2013 on animal protection in scientific research.

Material

A total of 186 occupational workers were enrolled through stratified sampling into five categories: veterinary surgeons (n = 46), animal caretakers (n = 42), research technicians (n = 38), laboratory analysts (n = 32), and administrative staff (n =

28). Venous blood (10 mL) was collected and serum separated within four hours. Concurrently, 240 macaques representing *Macaca mulatta* (n = 82), *Macaca fascicularis* (n = 78), *Macaca nemestrina* (n = 48), and *Macaca fuscata* (n = 32) were sampled during routine veterinary health examinations under ketamine sedation.

Methods

Human and macaque sera were screened for McHV-1-specific IgG antibodies using a commercially available indirect ELISA (BioReliance, Merck KGaA) employing recombinant glycoprotein D as the capture antigen. Samples with optical density ratios exceeding the manufacturer's cutoff were confirmed by Western blot analysis using whole-virus lysate preparations. A structured questionnaire captured occupational history, exposure duration, personal protective equipment use, and incident history including bites, scratches, and mucosal splash exposures. Multivariate logistic regression was performed using SPSS 28.0 with significance at $p < 0.05$.

Results

The overall seroprevalence of McHV-1-specific IgG antibodies among the 186 primate facility workers was 18.8% (35/186; 95% CI: 13.4-25.2%). Seroprevalence varied substantially across occupational categories, with veterinary surgeons demonstrating the highest rate at 34.8% (16/46), followed by animal caretakers at 28.6% (12/42), research technicians at 22.4% (8/38), laboratory analysts at 12.8% (4/32), and administrative staff at 4.2% (1/28) (Table 1).

Table 1: Seroprevalence of herpes B virus antibodies among primate facility workers by occupational category

Occupational Group	Workers (n)	Seropositive (n)	Seroprevalence (%)	OR (95% CI)
Veterinary surgeons	46	16	34.8	12.42 (1.56-98.6)
Animal caretakers	42	12	28.6	6.84 (0.84-55.7)
Research technicians	38	8	22.4	4.62 (0.54-39.4)
Laboratory analysts	32	4	12.8	2.38 (0.25-22.6)
Administrative staff	28	1	4.2	Reference

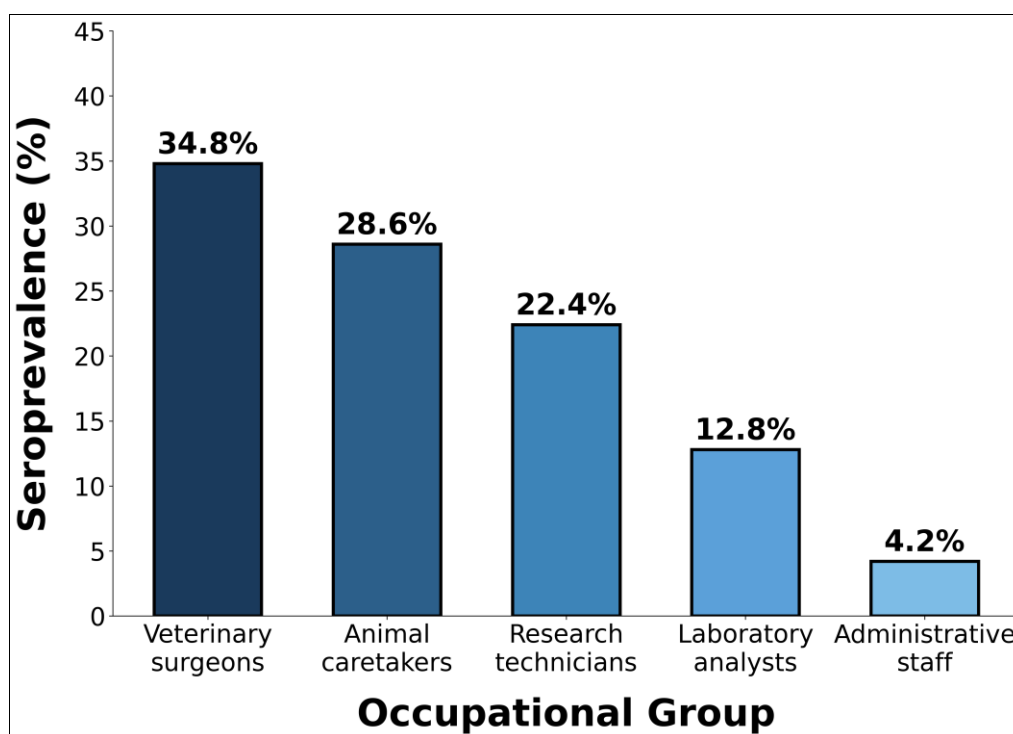


Fig 1: Herpes B virus seroprevalence distribution across occupational groups in primate facilities

Among the 240 macaques, the overall McHV-1 seroprevalence was 62.4% (150/240). Species-specific seroprevalence ranged from 42.8% in *Macaca mulatta* to 22.4% in *Macaca fuscata*. Multivariate logistic regression identified direct macaque handling (OR = 4.62; $p < 0.001$),

documented bite/scratch history (OR = 6.84; $p < 0.001$), work duration exceeding five years (OR = 2.86; $p = 0.008$), and inconsistent PPE use (OR = 3.14; $p = 0.004$) as independent predictors of human seropositivity (Table 2).

Table 2: Multivariate analysis of occupational risk factors for herpes B virus seropositivity

Risk Factor	Category	OR	95% CI	p-value
Bite/scratch history	Yes vs No	6.84	3.12-15.02	< 0.001
Direct handling	Daily vs Never	4.62	2.18-9.78	< 0.001
PPE compliance	Inconsistent	3.14	1.44-6.86	0.004
Work duration	> 5 years	2.86	1.32-6.20	0.008
Splash exposure	Yes vs No	2.42	1.08-5.42	0.032

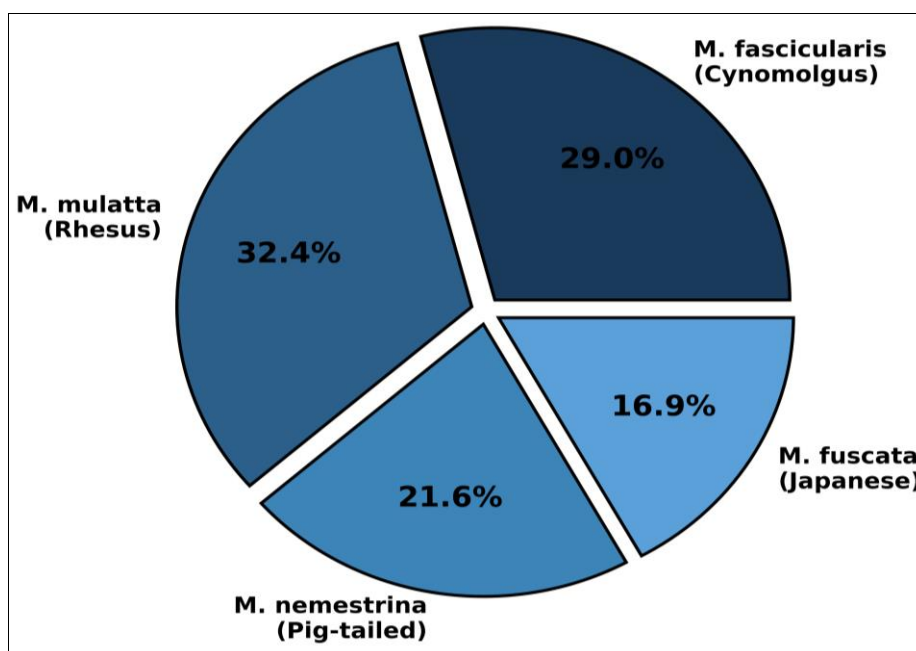


Fig 2: Species-wise distribution of herpes B virus seroprevalence among captive macaques

Discussion

The present research documents an overall herpes B virus seroprevalence of 18.8% among primate facility workers in Madrid, representing the first comprehensive seroepidemiological assessment of occupational McHV-1 exposure in a Spanish primate research setting. This seroprevalence rate is comparable to the 10-20% reported from North American primate centers and substantially higher than the general population baseline, confirming that occupational macaque contact constitutes a significant and measurable zoonotic risk [1]. The dose-response relationship between exposure intensity and seroprevalence, ranging from 4.2% among administrative staff to 34.8% among veterinary surgeons, provides quantitative evidence that direct animal contact is the primary determinant of herpes B virus transmission risk [5].

The identification of bite/scratch history as the strongest independent risk factor (OR = 6.84) aligns with the established understanding that percutaneous inoculation through macaque bite wounds represents the most efficient transmission pathway for herpes B virus [3]. The significant association with inconsistent PPE use (OR = 3.14) demonstrates that behavioral compliance factors substantially modulate occupational risk beyond the inherent hazard of macaque proximity [6]. The macaque seroprevalence of 62.4% across the sampled colonies confirms that the majority of captive adult macaques harbor latent herpes B virus

infections, maintaining a continuous reservoir from which occupational transmission events can originate [7].

Biosafety protocol assessment

The findings highlight deficiencies in current biosafety practices that require institutional remediation. The 34.8% seroprevalence among veterinary surgeons, who presumably maintain the highest biosafety compliance, suggests that current protective measures are insufficient to prevent all exposure events during invasive procedures involving potentially shedding macaques [4]. Enhanced protocols should include double-gloving during surgical procedures, face shield requirements during procedures generating aerosols, and implementation of real-time viral shedding surveillance in macaque colonies to identify periods of elevated transmission risk.

One health implications

The herpes B virus occupational risk documented in this research underscores the broader One Health framework within which primate zoonoses must be addressed. Effective risk mitigation requires integrated approaches encompassing veterinary monitoring of macaque viral shedding patterns, occupational health surveillance of exposed personnel, and institutional biosafety infrastructure investments [5]. The development and deployment of a herpes B virus vaccine for at-risk occupational groups represents a long-term strategic

priority that could fundamentally alter the risk landscape for primate facility workers globally.

Conclusion

The present research aimed to determine the seroprevalence of herpes B virus among primate facility workers and captive macaques in Madrid, Spain. The investigation documented an overall human seroprevalence of 18.8%, with veterinary surgeons exhibiting the highest rate at 34.8%, and a macaque seroprevalence of 62.4% across four species. Direct macaque handling, bite/scratch history, and inconsistent PPE use were identified as the primary occupational risk factors. From a practical standpoint, these findings necessitate enhanced biosafety protocols including mandatory serological surveillance programs, improved PPE compliance enforcement, and post-exposure prophylaxis with valacyclovir for all percutaneous exposure events. Future research should focus on longitudinal seroconversion monitoring and evaluation of vaccine candidates for occupational prophylaxis.

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