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### Seroprevalence and Risk Factors of Infectious Bovine Rhinotracheitis in Dairy Cattle of Chitwan, Nawalpur and Rupandehi Districts of Nepal

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#### KEYWORDS

Infectious Bovin

Rhinotracheitis

Seroprevalence

Risk factors

Dairy cattle

#### ABSTRACT

The cross-sectional study from July 2018 to September 2018 was conducted to determine the seroprevalence and risk factors of Infectious Bovine Rhinotracheitis (IBR) in cattle of the Chitwan, Nawalpur, and Rupandehi districts of Nepal. The existence of antibodies against IBR was investigated in 92 serum samples obtained systematically from 55 cattle herds using Indirect-ELISA. A questionnaire interview was done to collect individual and herd-level data. The association between categorical variables and the outcome variable (seropositive) was assessed by bivariate analysis and multivariate logistic regression analysis in SPSS version 19.0. The seroprevalence of IBR was 18.48% (95% CI: 11.1-27.9), and district, breed, and herd size were identified as potential risk factors for IBR seropositivity. Significantly higher risk for IBR was found in Chitwan (Percentage-Positive “PP” = 36.37%; Odd ratio “OR” = 5.211; p = 0.008) than in Nawalpur (PP = 9.38%; OR = 0.931) and Rupandehi (PP = 10.00%). PP of IBR was significantly higher in Jersey crosses (PP = 30.00%; OR = 2.893; p = 0.048) than Holstein Friesian crosses (PP = 12.90%). Similarly, herds with more than 10 cattle (PP = 33.33%; OR = 4.167; p = 0.042) were found significantly at higher odds for seropositivity

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than herds having less than 10 cattle (PP = 10.71%). Due to the moderate prevalence of IBR among cattle in Nepal, this study recommends conducting additional planned research on IBR at the national level to safeguard the country's dairy businesses from potential financial losses.

## 1 Introduction

In recent years, decreased fertility in dairy cows has become an international issue. The reproductive process of dairy cows is hampered by several factors. The main contributor to infertility in dairy cattle is infectious agents such as bacteria, viruses, and parasites (Prakash et al. 2021a; Prakash et al. 2021b; Chandran et al. 2021a; Chandran et al. 2021b; Sharun et al. 2021; Tiwari et al. 2022). These agents can lead to estrus, repeated pregnancy, early embryonic death, retention of placenta, delayed return to estrus, early embryonic death, and abortion. These will eventually trigger calving and a decrease in milk production in dairy farms (Chandran et al. 2019; Sonaa et al. 2021; Kumari et al. 2022).

Bovine herpes virus-1 (BoHV-1) causes infectious bovine rhinotracheitis (IBR), also known as a red nose or necrotic rhinitis, which is a highly contagious, infectious viral disease of cattle (Muylkens et al. 2007; Nandi et al. 2009), resulting in significant economic losses for the dairy and beef industries due to abortions, metritis, retention of placenta, repeat breeding, death of animals, loss of production and trade restrictions (Verma et al. 2014; Chandran and Arabi 2019; Chandran 2021a; George et al. 2021; Anand et al. 2022). While bovines are the most common hosts for BoHV-1, the virus has been found in other Artiodactyls (including goats, sheep, water buffaloes, and camelids) as well (Biswas et al. 2013). The main route of transmission is by direct contact with nasal, ocular, and genital secretions from infected animals (Ackermann et al. 1990; Nuotio et al. 2007), or indirectly through contaminated material and air-borne particles (Wentink et al. 1993). The disease is clinically manifested by respiratory signs like serous nasal discharge, salivation, conjunctivitis, fever, anorexia, and depression (Ackermann et al. 1990; Miller 1991; van Oirschot et al. 1996). Pustular vulvovaginitis and balanoposthitis are two genital infections that commonly occur in areas where natural mating is common (OIE 2017). Most of the infections have a very moderate or subclinical course, but some can result in the abortion-inducing evacuation of a dead fetus and bloody fluid, as well as reproductive abnormalities such as repeat breeding, metritis, and retention of the placenta (van Oirschot et al. 1993; Chandran 2021a; Chandran 2021b; Lejaniya et al. 2021a; Lejaniya et al. 2021b).

Trigeminal ganglia after a respiratory infection and sacral ganglia following genital infection are the primary sites of latency in IBR; other non-neural locations include lymph nodes in the pharynx, cervical region, retropharynx, inguinal, and tonsils (Winkler et al. 2000), as well as peripheral blood (Fuchs et al. 1999). Reactivation of the latent infection can occur under stressful settings such as

transport and parturition and through the use of corticosteroids, and viral DNA persists in ganglion neurons, likely for the lifetime of the host (Muylkens et al. 2007). Consequently, the virus may switch between a latent and lytic infection that can be identified through the detection of antibodies against BHV-1 in serum (Lemaire et al. 2000).

IBR is OIE listed notifiable disease and emerging disease of cattle (OIE 2017; Chandran 2021a), and it was first recorded in 1841 in Germany by Buchner and Reimann in the form of infectious pustular vulvo-vaginitis (IPV) in cattle (Biswas et al. 2013), IBR is now widespread all over the world except few countries like Austria, Denmark, Finland, Sweden, and Switzerland (Ackermann and Engels 2005). In Nepal, researchers have confirmed the seroprevalence of IBR (Jha 2005; Dyson et al. 2000). Out of 118 serum samples from cattle having reproductive problems like abortion, anestrus, and repeat breeding 60 samples i.e. 50.8%, were found seropositive for IBR in Nepal (Jha 2005). It was also discovered by Dyson et al. (2000) through research conducted in Nepal that animals exhibiting reproductive problems have antibodies against IBR. However, there is a lack of data in the study area because of the paucity of studies on the seroprevalence and risk factors related to IBR in cattle across the country. The purpose of this research was to assess the incidence of IBR and identify the factors that put cattle at risk for developing the disease in the farms of Nepal's Chitwan, Nawalpur, and Rupandehi districts, specifically among the population of improved cattle that has a documented history of reproductive issues (including abortion, placental retention, and repeated breeding).

## 2 Materials and Methods

### 2.1 Study area, design, and experimental animals

A cross-sectional descriptive study was conducted from July 2018 to September 2018 in 55 herds of improved dairy cattle from 20 different areas of Chitwan, Nawalpur, and Rupandehi districts of the mid-tropical region of Nepal (Figure 1), having an estimated cattle population of 71864, 61086 and 98384 cattle respectively (MoLD 2017). These districts possess a similar geographical background, climatic conditions, population density, vegetation, and biodiversity. People living in these areas are mainly involved in agriculture and animal husbandry. These districts are well-known in the country for dairy cattle farming and production. Thus, the areas were selected purposively as these areas have a larger number of cattle and commercial dairy farms in the country (Chandran 2021a; Chandran 2021b).

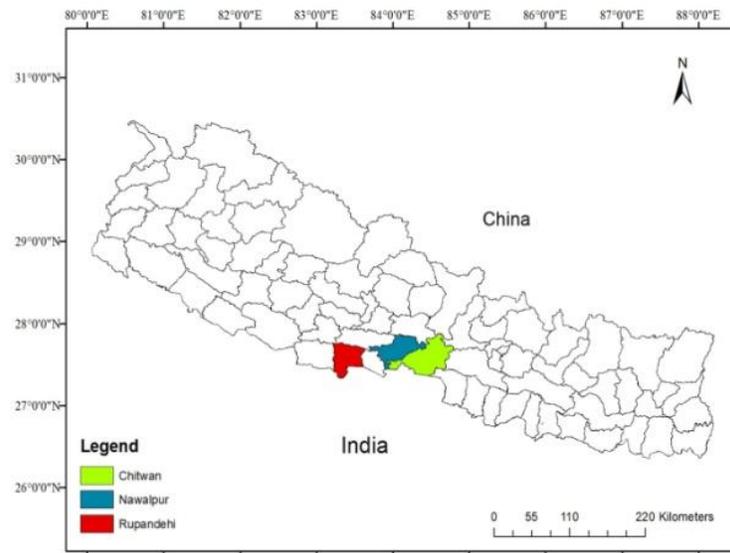


Figure 1 Map of Nepal showing the study area

The required sample size was 382, calculated from the EpiTools epidemiological calculator by Ausvet with an assumption of 50.8% prevalence of IBR in improved and crossbreed cattle of Nepal as reported by Jha (2005), keeping expected precision of 5% and 95% confidence level. However, due to the unavailability of an ELISA kit, only 92 samples were taken into consideration.

The animals used in the study were female dairy cattle of more than 2 years of age having a history of reproductive problems like abortion, retention of placenta, and repeat breeding. The animals were selected for sample collection by a purposive two-stage sampling procedure. In the first stage, pocket areas from each district (7 areas from Chitwan, 6 areas from Nawalpur, and 7 areas from Rupandehi) were selected by convenience sampling procedure which was taken as the primary sampling unit (PSU). In the second stage, blood samples of cows with a history of reproductive disorders were collected, by visiting the farms purposively in the preselected pocket areas, which were taken as a secondary sampling unit (SSU).

## 2.2 Variables

### 2.2.1 Dependent variable: Seropositivity for IBR

### 2.2.2 Independent variable

- Age: categories of age 2-5 years and  $\geq 5$  years
- Herd size: categories of  $\leq 10$  cattle and  $> 10$  cattle
- Breed: Holstein Friesian Cross and Jersey Cross
- History of reproductive problems like abortion, ROP, and repeat breeding.

## 2.3 Epidemiological Data

Epidemiological data were collected by using a structured questionnaire having both open-ended and close-ended questions for individual animal data as well as herd-level data. Individual level data included age, breed, pregnancy status and history of abortion, retention of placenta, and repeat breeding. Herd-level data include husbandry practices, vaccination, housing system, feeding system, interaction with other animals, the introduction of a new member, and breeding practice. The questionnaire was completed through face-to-face interviews with the farm owner.

## 2.4 Blood Samples

Selected animals were properly restrained, and 5 ml of blood was collected in a sterile SSGT (serum separating gel tube), properly labeled and immediately kept in a cool box. Then, the blood samples were brought to the laboratory of the National Cattle Research Program (NCRP), Chitwan, Nepal, where sera were extracted by centrifugation at 3000 rpm for 10 minutes. Extracted sera were then transferred into Eppendorf tubes, labeled, and stored in a deep freezer at  $-20^{\circ}\text{C}$  until laboratory analysis was performed.

## 2.5 Laboratory Analysis

Serum samples were tested by Indirect ELISA manufactured by IDvet, France (ID Screen® IBR Indirect) for the detection of anti-BOHV-1 antibodies. The test was conducted in the laboratory of the National Cattle Research Program (NCRP), Rampur, Chitwan, Nepal as per the protocol and procedures provided by the kit manufacturer.

## 2.6 Statistical Data Analysis

Data were first coded in the MS Excel spreadsheet (MS Office 2013) and then imported into SPSS version 19.0 for statistical analysis. The overall prevalence rate and district-wise prevalence and 95% confidence interval for prevalence were calculated from SPSS. Similarly, the association between categorical variables and the outcome variable (seropositive) was assessed by bivariate analysis (Pearson chi-square test) in SPSS. Finally, the multiple effects between predicted variables and outcome variables were analyzed by the forward likelihood ratio (Forward LR) method in a logistic regression model. For all analyses, a p-value of less than 0.05 at 95% CI was considered statistically significant. Finally, tables were used to present the results generated from SPSS.

## 2.7 Ethical Consideration

The study was conducted following the Declaration of Helsinki and approved by the Internship Advisory Committee of Veterinary Teaching Hospital, Institute of Agriculture and Animal Science, Tribhuvan University, Nepal. Oral consent was sought from

farmers before commencing blood sampling from each farm or herd. The pain was kept to a minimum during blood collection.

## 3 Results

### 3.1 Overall seroprevalence

As shown in table 1, out of 92 serum samples tested, 17 samples were found positive for IBR with an overall seroprevalence of 18.48% (95% CI: 11.1-27.9%). Among them, 11, 3 and 3 samples were positive from Chitwan, Nawalpur and Rupandehi out of 30, 32 and 30 samples collected, resulting seroprevalence of 36.37% (95% CI: 19.9-56.1%), 9.38% (95% CI: 2-25%) and 10.00% (95% CI: 2.1-26.5%) respectively (Table 1).

### 3.2 Risk factors associated with IBR Seropositivity

Bivariate analysis (using Pearson chi-square test) between categorical variables (risk factors) and the outcome variable (IBR seropositivity) (Table 2) revealed a statistically significant association of IBR with location, breed, and herd size ( $P < 0.05$ ),

Table 1 Overall Seroprevalence of IBR in Cattle of Chitwan, Nawalpur, and Rupandehi District based on SPSS

Test Assay	District (Total Samples)	Classification	Prevalence %	95% CI
ELISA	Chitwan (30)	Positive:11 Negative:19	36.7	19.9-56.1
	Nawalpur (32)	Positive:3 Negative:29	9.4	2-25
	Rupandehi (30)	Positive:3 Negative:27	10.00	2.1-26.5
Total	92	Positive:17 Negative:75	18.5	11.1-27.9

Table 2 Results of Bivariate Analysis of Risk factors associated with seroprevalence of antibodies of IBR

Factors	Categories	N	Seropositive N (%)	$\chi^2$ Value	P-Value	Odd's ratio (95% CI)
Location	Chitwan	30	11(36.37)	9.780	0.008*	5.211(1.278-21.237)
	Nawalpur	32	3(9.38)			0.931(0.173-5.015)
	Rupandehi	30	3(10)			Ref.
Breed	HF Cross	62	8(12.90)	3.923	0.048*	2.893(0.985-8.498)
	Jersey Cross	30	9(30.00)			
Herd size	>10	27	9(33.33)	4.123	0.042*	4.167(0.987-17.592)
	$\leq 10$	28	3(10.71)			
Age	2-5	39	4(10.26)	3.038	0.081	2.844(0.849-9.527)
	$\geq 5$	53	13(24.53)			
Abortion history	Yes	26	5(19.23)	Fisher's exact test	1.000	
	No	66	12(18.18)			
Retention of placenta history	Yes	24	2(8.33)	Fisher's exact test	0.221	
	No	68	15(22.06)			
Repeat breeding history	Yes	62	11(17.74)	0.068	0.794	
	No	30	6(20.00)			

\*: Statistically significant; CI: Confidence Interval

Table 3 Results of Logistic Regression analysis for IBR seropositivity

Variables	N	Odd's Ratio	95% CI	p-value
Location	92			
Chitwan	30	4.209	0.929-19.077	0.009
Nawalpur	32	0.398	0.062-2.546	
Rupandehi	30	Ref	-	
Herd Size (> 10/≤10)	92	6.615	1.506-29.061	0.012
Breed (HFC/JC)	92	0.256	0.070-0.935	0.039

CI: Confidence Interval; HFC: Holstein Friesian Cross; JC: Jersey Cross

whereas age and history of reproductive disorders like Abortion, ROP and Repeat breeding were not significantly associated ( $P>0.05$ ). Again, in the logistic regression model using the Forward LR method with 95% CI, the association of the seroprevalence of BoHV-1 infection was analyzed for the predicted variables (variables with  $P<0.20$  in bivariate analysis), which identified location ( $P=0.009$ ), herd size ( $P=0.012$ ) and breed ( $P=0.039$ ) as risk factors (Table 3).

### 3.3 Breed-wise seroprevalence

Out of 30 samples from the Jersey cross (JC) and 62 samples from the Holstein Friesian cross (HFC) 9 (30.00%) and 8 (12.90%) samples were found positive respectively. Seroprevalence of IBR between JC and HFC breeds was found significant ( $P<0.05$ ) where Jersey cross-breed cattle had higher odds to be seropositive than HF cross breeds (OR=2.893; 95% CI: 0.985-8.498) (Table 2).

### 3.4 Herd-wise seroprevalence

Among 55 herds tested, 28 herds were having cattle  $\leq 10$  and 27 herds were having cattle  $>10$ , out of which 3 (10.71%) and 9 (33.33%) herds were found seropositive for IBR respectively (Table 2). The seropositive herd was the herd with at least one seropositive animal. Seroprevalence of IBR between herd sizes of  $\leq 10$  and  $>10$  was significant ( $P=0.042$ , i.e.  $P\leq 0.05$ ), showing higher odds in herds having more than 10 cattle to the ones having less than or equal to 10 cattle (OR=4.167; 95% CI: 0.987-17.592)

### 3.5 Age-wise seroprevalence

Cattle below 2 years of age were not included in this study. Four samples out of 39 samples from age groups 2-5 years and 13 samples out of 53 samples from  $\geq 5$  years of age were found positive (Table 2) with a seropositive percentage of 10.26% and 24.53% respectively. There was no significant difference between seropositivity and age categories of 2-5 years and  $\geq 5$  years ( $P>0.05$ ).

### 3.6 Seroprevalence by reproductive problems

The medical history of the abortion, retention of placenta, and repeat breeding were found in 28%, 26%, and 67% of the total

cattle sampled respectively. Five samples out of 26 samples had abortion history, 2 samples out of 24 samples had retention of placenta (ROP) history, and 11 samples out of 62 samples having repeat breeding history were found positive for IBR (Table 2) with seropositivity percentage of 19.23%, 8.33%, and 17.74% respectively. There was no significant association between the seroprevalence of IBR and any of the history of reproductive problems like abortion, ROP, and repeat breeding ( $P>0.05$ ).

## 4 Discussion

In the present study, the overall prevalence was found to be 18.48% (Total samples=92) which is similar to the prevalence found in Meru, Kenya (17.4%) (Kipyego et al. 2020), Karnataka (21%) (Koppad et al. 2007), Uttaranchal (10.75%) (Jain et al. 2006), Kerala (14.88%) (Rajesh et al. 2003) and West Bengal was (22%) (Ganguly et al. 2008), but, lower than the previous finding reported in Nepal by Jha (2005) i.e., 50.8% (Total samples=118) and study in Turkey (39.53%) (Ince and Şevik 2022). Tests conducted on animals in Nepal that were exhibiting reproductive abnormalities revealed the existence of antibodies against IBR, as was previously reported by Dyson et al. (2000). According to these studies, the BoHV-1 virus is widespread in Nepalese cattle farms. However, since there is no evidence of vaccination practice against IBR in Nepal as of yet, the circulating antibodies are likely only the result of natural infection of the virus already present within the herd or from newly introduced infected cattle without proper screening (Kampa et al. 2004). However, the low seroprevalence of IBR in this study may be mainly because of two reasons: first, a fall of immune response below detection after some years (OIE, 2017) as self-clearance of the virus may occur over time by replacement of infected and imported animals which is further potentiated by probably low intensive production systems i.e. with a low level of stress to animals (Kampa et al. 2004), and second is due to small but significant seasonal association of IBR (winter is a high risk than summer for IBR), detected by Woodbine et al. (2009) and supported by Sayers et al. (2015), and the present study was done in summer (July to September is summer in Nepal). Also, in contrast to our result, higher seroprevalence was found in different areas like 14%–60% in Africa and 36%–48% in Central and South America (Straub, 1990), 36% in China (Yan et al., 2008), 43% in England (Woodbine et al., 2009), and 63%–86% in

Egypt (Mahmoud et al., 2009). Similarly, in Great Britain, among unvaccinated herds of cattle, the prevalence was found to be 62% by the bulk milk tank test (Martina et al. 2017). According to Kampa et al. (2004), variation in the prevalence rate of IBR at various farms is likely caused not only by the reactivation of dormant BoHV-1 infection under natural conditions but also by variances in management and/or geographical variables. Also, a study that was based on medical history concluded that non-vaccination, intensive rearing, the purchase and mixing of animals without IBR screening in all of the farms, and natural insemination from unscreened bulls in one farm are thought to be the primary causes of the high prevalence (Patil et al. 2015).

In location-wise analysis, Chitwan district has significantly higher seroprevalence (36.37%;  $p < 0.05$ ) as compared to Nawalpur (9.38%) and Rupandehi (10.00%) in this study. The reason behind this may be the differences in management and population of cattle as Chitwan has almost double the number of improved cattle (24744) as compared to Nawalpur (12092) and Rupandehi (13175). A high density of dairy cows and intensive management promote the viral spread and increase the chances that healthy susceptible animals will encounter infected animals (Chandranaik et al. 2014). To a similar extent, there is a greater likelihood of migration of cattle from one herd to another in Chitwan, which may play a role in the transmission and consequently results in a higher prevalence of the IBR antibody among cattle in that region.

Out of 30 samples from the Jersey cross (JC) and 62 samples from the Holstein Friesian cross (HFC), 9 (30.00%) and 8 (12.90%) samples were found positive respectively. JC breeds showed significantly higher odds for seroprevalence of IBR in comparison to HFC breeds ( $P < 0.05$ ;  $OD = 2.893$ ; 95%  $CI: 0.985-8.498$ ) which is in contrast with Singh et al. (1985), who concluded that the prevalence was higher in Holstein-Friesian (50.35 %) than in Jersey (35.48 %) while screening a total of 506 cattle, and Sarmah et al. (2015), who screened only 51 samples of breeding bulls. The plausible explanation seems to be that most of the exotic cattle breeds reared in Nepal are Jersey Crossbreed as compared to Holstein Friesian which might reflect more transmission contact, thus higher prevalence.

Seroprevalence of IBR between herd size with cattle  $\leq 10$  (10.71%) and  $> 10$  (33.33%) was statistically significant ( $P < 0.05$ ) in this study which is in agreement with the previous finding by Woodbine et al. (2009) and Boelaert et al. (2005). Higher rates of seropositive animals in larger herds may be attributable to an increase in opportunities for disease transmission, both within and across herds (through, for example, veterinarians, technicians, workers, other farmers, and acquired cattle) (Woodbine et al. 2009; Boelaert et al. 2005; van Schaik et al. 1998). Reactivation of latent infections is also more likely to occur in larger herds due to the

added stress of having more animals and maybe introducing new calves into the herd (Singh and Sinha 2006).

For the age-wise analysis, blood samples from reproductively mature cattle ( $\geq 2$  years of age) were sampled and tested for IBR. There was no significant difference between seropositivity and age categories of 2-5 years and  $\geq 5$  years ( $P > 0.05$ ). However, 4 samples out of 39 samples from age group 2-5 years and 13 samples out of 53 samples from  $\geq 5$  years of age were found positive with seropositive percentages of 10.26% and 24.53% respectively. Increasing seropositivity with an increase in age was observed which is in accordance with the findings of Singh and Sinha (2006), Ganguly et al. (2008), Woodbine et al. (2009), Bandyopadhyay et al. (2009), Verma et al. (2014), Patil et al. (2015) and Samrath et al. (2016). Possible causes include animals becoming more susceptible to disease as they become older; recurring, low-grade infections with the same virus that keep the antibody titer high enough to be identified; or a combination of lowered immunity and greater stress that activates dormant viruses (Singh and Sinha 2006; Samrath et al. 2016).

Furthermore, the current study showed that 17(18.48%) among 92 samples with a history of reproductive disorders were seropositive for IBR antibodies. However, there was no significant difference between the seroprevalence of IBR and reproductive problems history like abortion, ROP and repeat breeding. Further, 5 out of 26 samples with abortion history, 2 out of 24 samples with ROP history, and 11 out of 62 sam repeat breeding history were found positive for IBR with seropositivity percentages of 19.23%, 8.33%, and 17.74% respectively. Seropositivity for IBR was discovered in 83% of abortion cases, 83% of repeat breeding cases, and 65% of retention of placenta cases, according to Patil et al. (2017), which is corroborated by studies conducted by Nandi et al. (2009) and Bera et al. (2015).

## Conclusion

In conclusion, this research study revealed that IBR is moderately prevalent among dairy cattle in Nepal. Statistical modeling revealed that geographic regions, breed, and herd size are the strongest indicators of IBR occurrence. Seroprevalence estimated in this study provides the basis for future monitoring and surveillance of disease with an urge for further planned research on IBR at the national level and to introduce the vaccination practice to protect our dairy industries from potential economic losses due to the infectious disease, despite the limitations of small sample size and a purposive sampling procedure.

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### Conflict of Interest

No authors declare a conflict of interest.

### Data Availability Statement

The datasets generated during and/or analyzed during the study are available from the corresponding author upon reasonable request.

### Author's Contribution

SP, and MPA conceptualize the study. SP, SS and MPA designed the methodology. SP, DS, and SS performed the field and laboratory work. SP, SS and DS wrote the original draft. SP, DS, MPA, DC, KD reviewed and edited the final manuscript. All authors read and approved the final manuscript.

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