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SURVEILLANCE FOR HEMORRHAGIC SEPTICEMIA IN BUFFALO (*BUBALUS BUBALIS*) AS AN AID TO RANGE EXPANSION OF THE JAVAN RHINOCEROS (*RHINOCEROS SONDAICUS*) IN UJUNG KULON NATIONAL PARK, INDONESIA

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ABSTRACT: The Javan rhinoceros (*Rhinoceros sondaicus*) of Ujung Kulon National Park (UKNP) is the crown jewel of Indonesia's rich natural history. The park lies on a peninsula surrounded by coastline and agriculture-dominated landscapes. The invasion of water buffalo (*Bubalus bubalis*) into the park carries a substantial health risk to the Javan rhinoceros and threatens plans to establish a new population outside of its only current range in UKNP. Hemorrhagic septicemia (HS), known locally as septicemia epizootica and caused by *Pasteurella multocida* B:2, could thwart Indonesia's efforts to expand the range of the Javan rhinoceros. Because HS was considered eradicated from Banten Province, few preventative programs have been available to farmers. During June 2012–July 2013, biologic samples were collected from 770 water buffalo in 19 villages. Deep nasal swabs (n=85) were taken for bacterial culture and blood samples (n=770) were collected for serologic testing. No animals were positive on culture. The prevalence of antibody to *P. multocida* in this population was 1.8% (14 of 770 animals). A structured questionnaire was used to gather information about possible risk factors. Husbandry practices associated with presence of antibody in water buffalo included lack of a permanent area to house buffalo at night, low body condition score (=2), high body temperature (≥40 C), a history of clinical signs or sudden death in the previous year, and a grazing system that utilized significant forage inside the park. Antibody was not associated with sex, age, vaccination status, or season. Understanding HS disease dynamics in the buffalo adjacent to UKNP may improve the livelihoods of people and health of endangered rhinoceroses in this ecosystem.

Key words: *Bubalus bubalis*, hemorrhagic septicemia, Javan rhinoceros, *Rhinoceros sondaicus*, septicemia epizootica, Ujung Kulon National Park, Indonesia, water buffalo.

INTRODUCTION

The critically endangered Javan rhinoceros (*Rhinoceros sondaicus*) is one of the most threatened of all land mammals and is the rarest of the five rhinoceros species with an estimated population of 51 animals in a single population on the western tip of the Indonesian island of Java (Ujung Kulon National Park [UKNP] 2012). With the recent loss of a remnant population in Vietnam, the entire world's population of the Javan rhinoceros survives only in the UKNP (Brook et al. 2014). The Javan rhinoceros population is not growing and perhaps declining.

A fundamental goal of the Indonesian Rhinoceros Conservation Action Plan is to create a second population of Javan rhinoceroses (DGFPNC 2007). A proposed range expansion into the Honje Mountains in eastern UKNP is the first step in meeting that goal. The Honje Mountain Range is nearly completely surrounded by 19 villages and the proposed range expansion would place rhinoceroses near humans and domestic animals. These rural agricultural communities are home to 44,518 Indonesians who rely on water buffalo (*Bubalus bubalis*; population of >3,600) for draught power, milk, and meat (McGinley 2008). Water buffalo and Javan rhinoceroses share the same UKNP forest

resources. Thirteen stock pens have been established in habitat zones inside UKNP that, according to footprint and camera trap data, are shared with at least eight Javan rhinoceroses (Fig. 1; WWF 2014).

Ujung Kulon National Park is geographically isolated at the west end of Java Island and is highly vulnerable to natural disasters, human pressure, inbreeding, irregular water resources, and diseases linked to past Javan rhinoceros deaths—hemorrhagic septicemia, anthrax, and trypanosomiasis have all been implicated in rhinoceros mortality (Mohamed et al. 2004; Rachmat et al. 2011). An increasing number of diseases of wildlife in national parks are likely to originate from contact with humans, domestic pets, or livestock within parks and in surrounding gateway communities (Deem et al. 2001; Gillin et al. 2002). Many infectious diseases currently threaten wild animal populations and biodiversity conservation (Daszak et al. 2001, 2004). Disease outbreaks and failure to prepare for preventable health risks may represent a barrier to expansion of the Javan rhinoceros population within and beyond the park.

In 1982, hemorrhagic septicemia (HS), caused by *Pasteurella multocida* B:2, was implicated in a Javan rhinoceros mortality event in UKNP (Carter and Chengappa 1981; DPNPI 1982; Schenkel 1982). Alarmingly, periodic rhinoceros deaths continue in UKNP with the loss of at least two rhinoceroses in 2002–03, five in 2010–13 (UKNP 2012), and two in 2014 (UKNP 2014). Disease transmission from syntopic buffalo and other livestock represents one plausible hypothesis for these mortalities because rhinoceros horns were intact with no evidence of poaching (UKNP 2012).

Fatal HS in cattle caused by *P. multocida* is known locally as septicemia epizootica (DPNPI 1982). Hemorrhagic septicemia also infects yaks (*Bos* sp.), camels (*Camelus* sp.), and water buffalo in a wide range of countries, including Indonesia. During the 1982 rhinoceros mortality, HS was implicated in outbreaks of disease in the water buffalo population bordering UKNP, though the diagnosis was later discounted because deaths in wild cattle (banteng; *Bos javanicus*) were not observed (Schenkel 1982). After the 1982 die-off, a government-sponsored HS vaccination program in the two subdistricts of our study area was intermittent. At the time of this study, the UKNP region was considered HS-free.

We measured the prevalence of HS in the water buffalo population in the 19 villages adjacent to UKNP and identified possible risk factors associated with HS cases. A better understanding of HS in the region's water buffalo may help guide disease control programs around the park while alerting conservation managers to the significant and preventable risks facing Javan rhinoceroses living near human settlements.

MATERIALS AND METHODS

Study area

The study site encompassed the 19 villages surrounding the Honje Mountains (6°38′5.48″–6°51′25.26″S, 105°29′46.33″–105°42′1.59″E) in the eastern portion of UKNP, Pandeglang District, Banten Province, Java, Indonesia. The buffer villages are divided into two subdistricts based on local government management authority: Sumur subdistrict in the west and Cimanggu subdistrict in the east (Fig. 1). Division of the study population into subdistricts followed political and administrative management authority. The Livestock and Animal Health Services of Pandeglang District manages the region in separate subdistricts and, importantly, the vaccination program is implemented by subdistrict. Study approval was granted by the headman of each subdistrict.

Study population, disease monitoring, and case definition

This study was approved by the Cornell University Institutional Animal Care and Use Committee (protocol 2006-0170). A cross sectional design with 95% confidence level and 50% prevalence was chosen because no previous data on prevalence were available in Indonesia. A sample size of 384 animals was calculated using Win Episcope 2.0 (Thrushfield et al. 2001) with an assumed water buffalo population of 3,600 (data from Cimanggu and Sumur subdistricts from the Livestock and Animal Services Pandeglang District in 2009). The calculated sample size

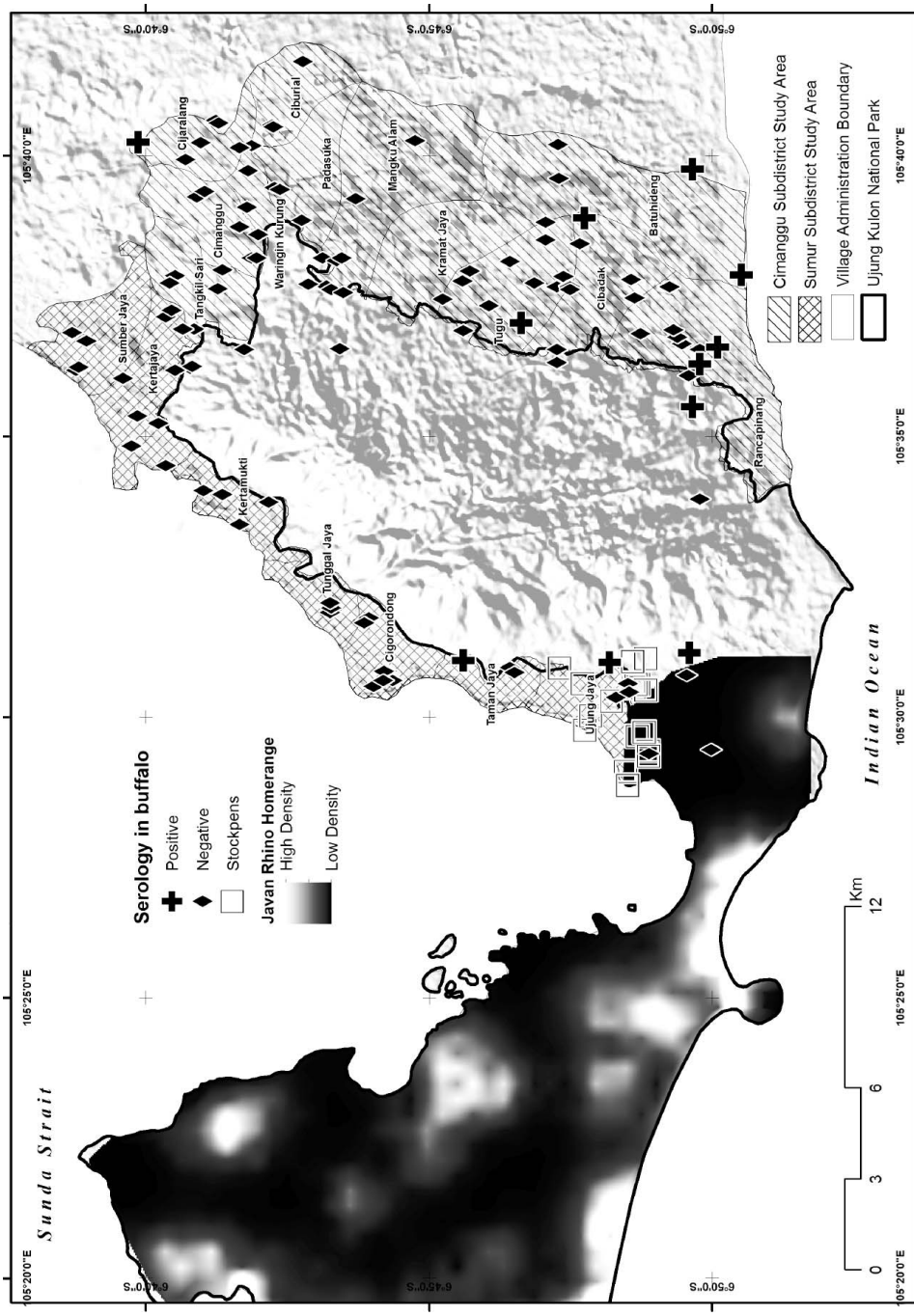

FIGURE 1. Map of a portion of Ujung Kulon National Park (UKNP), Indonesia and adjacent area showing the overlap of habitat ranges for water buffalo (*Bubalus bubalis*) and Javan rhinoceros (*Rhinoceros sondaicus*) and results of screening for antibody to *Pasteurella multocida*, which causes hemorrhagic septicemia in buffalo. A positive serologic result in water buffalo in the buffer villages surrounding the eastern portion of UKNP is denoted with a cross; a negative result is indicated with a diamond.

was multiplied by two (=768 animals) because the sampling plan involved two stages: random selection of subvillages in a village followed by random selection of buffalo stock pens within the subvillage. Buffalo stock pens are places where buffalo congregate and are areas utilized by more than one owner. Biologic samples were collected from 770 buffalo during the rainy and dry seasons, June 2012–July 2013.

A case of HS was clinically identified by severe respiratory distress with nasal discharge and frothing from the mouth leading to recumbency and death. Animals with these signs were reported to the study team through contact with local government officers (OIE 2012). Specific arrangements were made for each village leader to contact the research team when a suspect case was observed.

Blood was collected monthly for serologic assay in each of the 19 villages; culture swabs for HS bacteriology were collected opportunistically on the first 85 water buffalo. Culture of nasal swabs from cattle has inherent difficulties when the target organism is fastidious, as is *Pasteurella*, and likely to be overgrown by resident microbial flora. Therefore, we established a "case definition" rather than relying on culture results to confirm presence or absence of HS in water buffalo (Rovid Spickler et al. 2010).

Sampling

Blood samples were collected from water buffalo by venipuncture of the jugular vein with a 21-ga needle (BD Vacutainer® Eclipse™ Blood Collection Needle, Becton and Dickinson Company [BD], Franklin Lakes, New Jersey, USA) using a Vacutainer holder (BD Vacutainer® Holders, BD) and placed into clot tubes (Red BD Vacutainer™ Serum, BD). Blood in the tubes was allowed to clot and sera were transferred to cryovials (VMR®Cat, VMR International LLC, Radnor, Pennsylvania, USA) and stored frozen for a maximum of 7 d until transported to the laboratory. Samples were analyzed in Balai Besar Veteriner Denpasar-Disease Investigation Center (Bali, Indonesia) by enzyme-linked immunosorbent assay (ELISA) using an ELISA reader (Thermo Scientific Multiskan® Ex, Thermo Scientific, Wilmington, Delaware, USA).

Nasal swabs were collected using Pur Wraps® sterile polyester-tipped applicators (VMR International LLC). The applicator was placed into anaerobe transport medium (Anaerobe System Company, Morgan Hill, California, USA). Samples were held for a maximum of 5 d at room temperature (20–25 C). *Pasteurella multocida* dies shortly after storage at low temperatures (4 C); the optimal temperature for *P. multocida* is room temperature (Carter 1983). Samples were analyzed in the Unit Pelayanan Mikrobiologik Terpadu (an integrated microbiology service), Department of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agriculture University (Darmaga Bogor-Indonesia).

Pasteurella multocida culture

Isolates were identified based on morphologic and biochemical characteristics as described in the standard bacteriologic methods (Carter 1984). Isolates were identified as *P. multocida* using criteria of Holt et al. (1994). Culture samples were inoculated onto blood agar and MacConkey agar media. Plates were incubated at 37 C for 24–48 h in 5–10% CO_2. Smears were prepared from representative colonies (grey, viscous, and non-hemolytic) and microbes were characterized microscopically by Gram stain. Microscopic appearance of *P. multocida* was as coccobacilli or small rod-shaped Gram-negative cells occurring singly or in pairs or short chains. Bipolar staining was common with or without capsules.

Biochemical tests were performed for all isolates. Peptone water–grown culture of each isolate was inoculated into a 1% solution of glucose, sucrose, sorbitol, mannitol, fructose, dulcitol, lactose, salicin, arabinose, and maltose and incubated aerobically at 37 C for 24–48 h. Indole, oxidase, catalase, urease production, and nitrate reduction tests were carried out according to standard bacteriologic procedures (Carter 1984).

Serology

An ELISA was performed to detect antibodies to *P. multocida* with a sensitivity of 87.9% and specificity of 92.1% (Natalia and Priadi 1999). Our method followed Natalia et al. (1992): after checkerboard titration to determine the optimum antigen concentration and conjugate dilution, *P. multocida* antigen preparations were added to microtiter plates. Aspirated solution from wells was washed three times with phosphate-buffered saline and 0.05% Tween®20 (wash buffer). Serum samples (diluted 1:100 in Tris-EDTA-NaCl [TEN]-tween-casein 0.2%) were added to rows 1 through 10, with positive and negative controls added to rows 11 and 12. Samples were incubated at 37 C for 1 h and washed again three times with the same washing buffer. The conjugate anti-bovine immunoglobulin G previously labeled with horseradish peroxidase and diluted 1:3,000 with TEN-tween 0.2% casein was added into the plate well up to 100 μL and incubated for 1 h at 37 C. The plates were washed three times and 100 μL of the substrate buffer 2,2′-Azino-bis was added. After color developed, plates were read at 405 nm.

TABLE 1. Categoric classification of the risk factors for hemorrhagic septicemia (HS) in water buffalo (*Bubalus bubalis*) that border Ujung Kulon National Park (UKNP), Indonesia. BMKG = Indonesian Agency for Meteorology Climatology and Geophysics.

Risk factor	Definition
Body condition score (Ezenwa et al. 2009)	Score 2: All ribs visible with ridged feel, individual spinal vertebrae clearly palpable, points of hips protrude, flanks are concave, tail base visibly protrudes above surrounding tissue, and large bald patches across torso
	Score 3: Some ribs visible in center of ribcage, abdominal ribs have noticeable ridges, spine palpable as a slightly elevated bony centerline, points of hips distinctly visible, bone easy to feel but not protruding, tail base protrudes slightly, obvious by touch but not by sight, some bald patches behind the shoulders or along the flanks
	Score 4: Few ribs visible towards abdomen, ribs can be felt, spine bones not visible, spine feels flat, bone and surrounding tissue are level, hip bones have a round and smooth appearance, tail base level with surrounding tissue, thin coat covering entire body, glossy coat with few small bald patches
Age (Lendhanie 2005)	Subadult <3 y old; adult ≥3 y
Body temperature (FAO 1994)	No fever <40 C; fever ≥40 C
History of HS clinical signs <1 y	History of HS clinical signs in previous year (high fever, respiratory distress, nasal discharge, frothing from mouth)
History of sudden death <1 y	History of animal deaths during the previous year within herd
Grazing system	Outside: grazing areas for water buffalo are outside the UKNP
	Inside: grazing areas for water buffalo are inside the UKNP
Permanent area	No: owner does not have a permanent area to restrain buffalo during the night, or they have such an area for <6 mo
	Yes: owner has permanent area to restrain buffalo during the night for a minimum of 6 mo.
Season	Dry season: May–September (BMKG 2011/12)
	Rainy season: October–May (BMKG 2012/13)
Vaccination status	No: buffalo did not receive HS vaccine during previous year
	Yes: buffalo did receive HS vaccine during previous year

The ELISA results were recorded in ELISA units and samples with a titer ≥88 ELISA units were considered positive.

Questionnaire for risk analysis

The owners of water buffalo sampled during the study were interviewed using a standardized oral questionnaire. We collected basic health and husbandry information focusing on animal management practices believed to be risk factors for disease transmission in the UKNP region. The questionnaire was validated by confirming, when possible, information from independent sources that allowed for verification of buffalo management and husbandry data. For example, vaccination history of buffalo was compared with information received from the public health officer.

Categoric classification of risk factors was determined using currently established definitions (Table 1). For those factors in which no defined level of risk is available (e.g., permanent area), the definition was based on field experience of the investigators. The oral questionnaire used to collect information about water buffalo management practices from buffalo owners was exempt from Institutional Review Board review (exemption 1011001799).

Statistical analysis

Antibody prevalence for *P. multocida* in water buffalo was calculated as the number of animals positive by ELISA divided by the number of animals sampled. Prevalences between the subdistricts were compared using Fisher's exact test (IBM SPSS version 21, IBM Corp., Armonk, New York, USA).

Risk factors for HS as defined in Table 1 were compared with the antibody prevalence in water buffalo in bivariate analyses using Fisher's exact

test. A classification tree (CART) algorithm (IBM Corp.) using 10-fold cross-validation was applied to develop a multivariate model relating the meaningful risk factors identified in the bivariate analyses to positive *P. multocida* serology in water buffalo. Exact logistic regression (Stata version 12.1, StataCorp, College Station, Texas, USA) analysis was then performed to clarify the results obtained by CART.

RESULTS

Only three animals with positive clinical signs were found during the study. Clinical signs from these positive animals included severe frothing from the mouth and high fever followed by death. Nasal swab and serum samples were obtained from two of the three animals with clinical signs; one died before samples could be collected. Bacterial culture from both animals was negative, but the ELISA was positive in each case.

No samples were positive by bacterial culture and isolation. Of the 85 animals sampled for bacterial culture, only two demonstrated clinical signs of HS, and the remaining samples were collected from random, apparently healthy animals.

Fourteen serum samples were ELISA-positive. The mean antibody prevalence was 1.8% (14 of 770 animals, 95% confidence interval: 1.1–3.0%) (Table 2); 1.6% of Cimanggu subdistrict and 2.3% of Sumur subdistrict buffalo were positive. Prevalence did not differ between subdistricts (Fisher's exact $P=0.55$).

Age, sex, vaccination status, and season were not associated with positive *P. multocida* serology in water buffalo in villages adjacent to UKNP ($P>0.05$). However, body condition score (BCS), body temperature, permanent area, grazing system, history of sudden death in herd, and HS clinical signs were associated with positive *P. multocida* serology ($P<0.05$; Table 3).

Figure 2 illustrates the CART analysis representing the presence of antibody to *P. multocida* within 1 yr (CART model $R^2=0.54$). The CART indicated that antibody-positive animals could be identified from physical exam findings and disease history. Animals having a herd history of HS clinical signs within the previous year and having the lowest BCS (level 2), can be categorized as at high-risk for positive serology to HS (all 11 antibody-positive animals with a history of HS clinical signs also had a BCS of 2). Animals with no history of HS clinical signs, but living in a herd that had experienced sudden death within the previous year, can be categorized as at high-risk for *P. multocida* antibody (all three antibody-positive animals without a history of clinical signs of HS had a history of sudden death in the herd during the previous year).

The CART results revealed an optimal tree via 10-fold cross-validation which was parsi-

TABLE 2. Results of enzyme-linked immunosorbent assay (ELISA) for antibodies to *Pasteurella multocida* in 770 serum samples from water buffalo (*Bubalus bubalis*) in 19 villages surrounding Ujung Kulon National Park, Indonesia.

Subdistrict and village	No. animals positive/tested	Antibody prevalence (95% confidence interval)
Cimanggu		
Batu Hideng	1/41	0.024 (0.004–0.126)
Cibadak	2/43	0.047 (0.013–0.155)
Ciburial	0/50	0
Cigorondong	0/39	0
Cijalarang	2[a]/59	0.034 (0.009–0.115)
Cimanggu	0/48	0
Kramatjaya	1/49	0.020 (0.004–0.107)
Mangku Alam	0/39	0
Padasuka	0/39	0
Rancapinang	3/50	0.060 (0.021–0.162)
Tangkil Sari	0/54	0
Tugu	0/27	0
Waringin Kurung	0/54	0
Total	9/553	0.016 (0.009–0.031)
Sumur		
Sumber Jaya	0/2	0
Taman Jaya	2/43	0.047 (0.013–0.155)
Tunggal jaya	0/20	0
Ujung Jaya	3/43	0.070 (0.024–0.186)
Kertajaya	0/35	0
Kertamukti	0/35	0
Total	5/217	0.023 (0.01–0.053)
Overall	14/770	0.018 (0.011–0.03)

[a] Positive ELISA from animals showing clinical signs of hemorrhagic septicemia.

TABLE 3. Comparison of risk factors in water buffalo (*Bubalus bubalis*) that border Ujung Kulon National Park (UKNP), Indonesia and association with antibodies to *Pasteurella multocida* as detected by enzyme-linked immunosorbent assay (ELISA).

	Positive for *P. multocida* by ELISA		
Risk factor for hemorrhagic septicemia (HS)	No	Yes	Fisher's exact *P*-value
Body condition score			
2	202	13	<0.001[a]
3	467	1	
4	87	0	
Age			
Subadult	652	11	0.43
Adult	104	3	
Sex			
Female	709	12	0.22
Male	47	2	
Body temperature			
Fever	37	10	<0.001[a]
No fever	719	4	
HS vaccination status			
Vaccine	73	0	0.38
No vaccine	683	14	
History of HS clinical signs <1 y			
No	641	3	<0.001[a]
Yes	115	11	
History of sudden death <1 y			
No	559	1	<0.001[a]
Yes	197	13	
Grazing system			
Outside UKNP	461	3	<0.004[a]
Inside UKNP	295	11	
Permanent area			
No	288	10	<0.02[a]
Yes	468	4	
Season			
Dry	194	2	0.54
Rainy	562	12	

[a] Statistically significant difference.

monious and logical, though no *P*-values for statistical significance were produced using CART methodologies. To further validate the findings, an exact logistic regression model was fit that included the main effects of the three factors identified by CART (low BCS, a history of HS clinical signs, and a history of sudden death). This analysis supported the conclusion that buffalo with low BCS (level 2) had a significant association with positive serology (odds ratio [OR]=11.45, *P*=0.008), as did a history of HS clinical signs during the previous year (OR=4.83, *P*=0.030), and a marginally significant association for herds that had experienced sudden death within the previous year (OR=8.22, *P*=0.053).

DISCUSSION

The Javan rhinoceros is the flagship species of UKNP. The park lies on a peninsula surrounded by coastline and agriculture-dominated landscapes. The invasion of water buffalo into the park carries a substantial

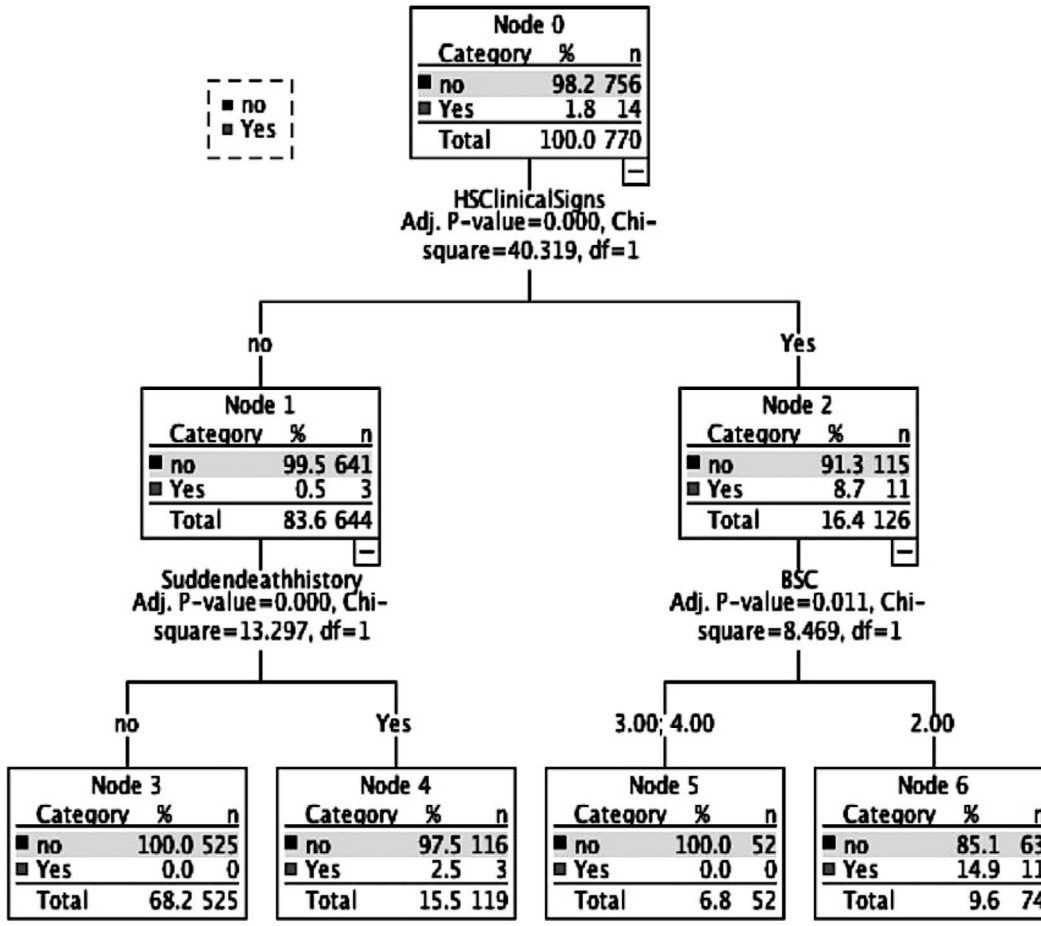

FIGURE 2. Classification tree (CART) algorithm for the presence of antibody to *Pasteurella multocida* in water buffalo (*Bubalus bubalis*) that border Ujung Kulon National Park, Indonesia. HS=hemorrhagic septicemia; BSC=body condition score.

health risk to the Javan rhinoceros and threatens plans to establish a new population outside of its only current range in UKNP. Hemorrhagic septicemia could threaten Indonesia's efforts to expand the range of the Javan rhinoceros. The first large-scale vaccination program for HS in Indonesia followed an outbreak in Banten Province in West Java (Natalia and Priadi 1998). The disease has since been considered eradicated and, consequently, the vaccination program in both subdistricts has been irregular and intermittent. This study revealed a low prevalence of *P. multocida* antibodies in water buffalo and suggests that carrier animals may lead to periodic epidemics of HS. Furthermore, risk factors related to animal husbandry and management practices were identified that could be changed and, if not, may threaten the health of syntopic Javan rhinoceroses.

The prevalence of HS in the water buffalo population in the Cimanggu and Sumur subdistricts was based on clinical signs and the detection of *P. multocida* antibodies by ELISA. Very few animals were observed with clinical signs characteristic of HS (only three animals during 1 yr of study). The paucity of observed clinical cases may be explained in several ways. First, clinical cases were likely missed because there was little knowledge among buffalo owners about the importance of good health management practices. There-

fore, the physical condition of animals was not regularly monitored. Second, the disease has a rapid course. Acute diseases with a short clinical period and rapid death are less likely to be observed by farmers. Lastly, geographic factors prevented more-comprehensive surveillance. Because both subdistricts cover large areas in a remote region, it was difficult for the research team to quickly respond to reports of HS. On 12 occasions the research team arrived after the animals died or had been sold. Buffalo owners typically sold sick animals or those in poor body condition as quickly as possible, making clinical case identification difficult. The geographic limitation of the study area may explain the underreporting of the disease to health officials—farmers in most cases were unaware that there was a disease reporting system available or that the official (only one government health official in the 19-village region) was interested in receiving reports of sick or dead animals.

The 85 negative culture results may represent animals not infected with *P. multocida* at the time of sampling. The two samples collected from animals with characteristic HS signs were also negative, possibly because the organism was not present in nasal secretions or the fastidious nature of the organism, together with overgrowth of abundant oral flora, precluded isolation (Rovid Spickler et al. 2010). Technical problems with sample collection or culture methods could also be responsible for the failure to detect *P. multocida* on culture. *Pasteurella multocida* is a fragile organism that could be overgrown by other competing bacteria. Ideally, sample collection should have been conducted aseptically by transtracheal wash; however, this was not practical in the field. The ideal sample for culture of *P. multocida* is a swab from the heart within a few hours of death of the animal (Muharsini et al. 2006). This technique was not utilized because sick or weak animals were often sold.

Of 14 animals positive by ELISA, only two simultaneously showed clinical signs of HS. Positive ELISA suggests that the animal has circulating antibodies. The two ELISA-positive animals showing typical HS clinical signs indicated they were currently infected with *P. multocida*. Positive serologic response in the absence of clinical signs could be interpreted two ways. First, the titer could represent a response to HS vaccination; however, according to the questionnaire none of the 14 ELISA-positive animals had been vaccinated during the previous year. Alternatively, the 12 ELISA-positive animals without clinical signs could have been naturally infected with *P. multocida* but recovered or did not develop clinical disease (e.g., were carrier animals). Because the vaccination program in both subdistricts has been irregular, the 1.8% antibody prevalence in this study likely represents a carrier state. The percentage of carriers for *P. multocida* can vary considerably, ranging from $<1\%$ to as high as 44% (Mohan et al. 1968).

The observations of De Alwis (1987) indicate that *Pasteurella* presence in the nasal passage is recurrent but transient and the organism can primarily be found in the tonsilar tissue. In our study, 11 of 14 antibody-positive animals lived in a herd with a history of clinical HS in the previous year. De Alwis (1982) concluded that a large proportion of buffalo in endemic areas harbor HS-causing pasteurellae in their tonsils and the organisms appear intermittently in the nasopharynx. Such animals also have high antibody levels (De Alwis 1992).

Carrier animals could play a role in outbreaks if various predisposing factors (physiologic and environmental) emerge in a population. Stress could cause pasteurellae harbored in the tonsils to proliferate and invade the nasopharynx and be disseminated via nasal secretions, infecting in-contact susceptible animals (De Alwis 1992). Stress is an outcome of many events such as poor nutrition due to food or water deprivation, adverse environmental conditions such as extremes in temperature, and poor husbandry such as overcrowding. Water buffalo in the UKNP region are managed under a loose and traditional husbandry style where animals are semi–free-ranging and suffer from variable nutrition and extremes in environmental conditions.

Carrier animals could endanger water buffalo and other susceptible animals that are syntopic. In this study, antibody-positive animals were predominately identified in the four villages closest to UKNP (Rancapinang, Cibadak, Taman Jaya, and Ujungjaya; Table 2 and Fig. 1). When a carrier animal is introduced into a new area with a highly susceptible population, an explosive outbreak could result (De Alwis 1992). In Indonesia where most diagnostic laboratories have limited capabilities, the ELISA is the most-commonly used diagnostic test for HS; as a screening tool it could add to our understanding of HS dynamics including characterization of carrier states and predictors of clinical disease (Muharsini et al. 2006).

In our study, neither age, sex, vaccination status, nor season were associated with the *P. multocida* antibody-positive water buffalo around the UKNP ($P>0.05$). Several studies have shown that age and season were related with the presence of HS (De Alwis et al. 1990; De Alwis 1992). We found that antibody-positive status in water buffalo was associated with husbandry factors such as the farmers' use of a permanent holding area and the grazing of animals inside the park. A permanent area (defined as buffalo having access to a permanent area where animals are restrained during the night; Table 3) seems to be protective against HS. Buffalo without a permanent area move from place to place, increasing the risk of contracting HS from other herd members. *Pasteurella multocida* can survive in the environment longer when outside the host. It can also survive for hours or possibly days in damp soil or water (OIE 2012). The presence or absence of a permanent area is also related to grazing area—water buffalo that graze inside UKNP are the same buffalo that do not have access to a holding area during the night ($P<0.001$).

Other factors associated with *P. multocida* antibody-positive status in UKNP buffalo include BCS, history of HS clinical signs in the herd during the previous year, and sudden death in the herd during the previous year. Hiramune and De Alwis (1982) indicated that the carrier state may be correlated with current exposure to HS. A history of HS clinical signs was associated with sudden death in the year preceding our study, suggesting that survivors of HS outbreaks may remain carriers and lead to further outbreaks.

Neither culture nor serology is a reliable measure of the true prevalence of HS. Due to the high mortality of buffalo with clinical HS in Indonesia, both culture and serology underestimate the prevalence of HS; we did not sample all animals infected because many had died before we could sample. Indonesian studies of HS in water buffalo report a morbidity rate (incidence) of 60% and a mortality rate near 100%; these data are not specific to the subdistricts around UKNP and instead represent rates on a national scale (Tarmudji 2003). One weakness of animal health management in Indonesia is incomplete epidemiologic data on many diseases, especially in rural regions such as around UKNP. Full recovery from clinical disease is known to occur only if the animal is treated in the very early stages of disease, which is impractical in this rural region (De Alwis 1999).

However, the ELISA is a valuable conservation tool because protected area managers are most interested in identifying carrier animals that have survived infection and therefore threaten the health of sympatric Javan rhinoceroses. If Javan rhinoceroses are susceptible hosts for *P. multocida*, then the presence of carrier buffalo in the villages closest to UKNP should serve as a warning to conservation planners as they design a second habitat and make significant conservation decisions for Javan rhinoceroses.

A key outcome of this study was the 2014 announcement from the local government (Livestock and Animal Health Services Pandeglang District) of a new HS vaccination program for the UKNP region that includes a dedicated budget for implementation of the program on an annual basis to make it sustainable. This program could have direct impact on domestic and wild animal health in the region, especially for livestock owners because their animals will be vaccinated at no expense to them. A vaccinated and well-managed buffalo population around UKNP

will minimize disease spread to the Javan rhinoceros within the park.

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