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SHORT COMMUNICATION

Veterinary aid clinic assessments of working ponies in West Nusa Tenggara province, Indonesia: A retrospective study

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ABSTRACT. Working ponies in the West Nusa Tenggara (NTB) province of Indonesia are relied upon as the principal mode of transport. They have important cultural, logistical and One Health significance for the local community. Given the tropical climate, these ponies face well recognised health and welfare challenges . Parameters relating to the general health and welfare of the ponies were assessed following data analysis of clinical records from three veterinary clinics held in 2018 and 2019. Records relating to 454 clinical examinations of ponies (n=365 stallions) aged between 1 to 25 years (mean 7.59 ± 4.70) were analysed. The mean body condition score (1 to 5 scoring system) across all clinics was 2.89 (±0.49; range 1.5, 4.5), with no significant difference between clinics (P= 0.297). The majority of ponies (84.57%; 95% CI 80.50, 87.92; 307/363) assessed presented with tachypnoea, 37.24% presented with tachycardia (95% CI 32.78, 41.92; 159/427), 14.80% (95% CI 11.10, 19.46; 41/277) recorded rectal temperatures considered hyperthermic (>38.5°C), and 38.0% did not show obvious evidence of sweating (95% CI 32.21, 44.16; 95/250). Ponies examined at the April/May 2019 clinic were more likely to be considered hyperthermic (P=0.009) and/or presented with tachycardia (P<0.001), whereas ponies examined in the November 2019 clinic were more likely to present with tachypnoea (P=0.001). In general, the objective measures of body condition and health indices of these ponies were considered adequate. Some abnormalities relating to prolonged recovery following exercise whilst working were considered likely related to thermoregulatory stress. Parasite burdens were found to be low, no haemoprotozoan parasites were detected and median faecal egg count was zero. Measures to encourage cooling and greater frequent rest periods continued surveillance and monitoring the health of these ponies will result in both enhanced welfare and advances in One Health initiatives.

Keywords: Ponies, Indonesia, thermoregulation, intestinal and blood-borne parasites, body condition score.

INTRODUCTION

The province of Nusa Tenggara Barat (NTB) in Indonesia comprises a cluster of islands in the southeast of the densely populated archipelago. The indigenous people of this region have traditionally relied heavily on ponies (predominantly breeds under <142cm (14 hands)) for the transport of people and goods, in addition to significant recreational and cultural activities (Anonymous, 2020; Pinsky *et al.*, 2019). The role of working equids is well recognised in lower to middle income communities where their welfare generally impacts the livelihood of dependents (Burn *et al.*, 2010; Sturgeon, 2021). Welfare issues associated with equids working in warmer climates have been recognised as particularly detrimental (Burn *et al.*, 2010). Such impacts on welfare and working lives of these equids has important ramifications, including reliance on transport of people and goods are given their well-recognised One Health role in such communities (Sturgeon, 2021). Volunteer veterinary aid clinics are regularly provided by international and Indonesian veterinarians and volunteers.[1]

Working in tropical climates poses thermoregulatory challenges for horses due to high ambient temperatures and humidity. Reliance on evaporative cooling in horses is vital for exercise-induced heat production, hence high humidity compromises any cooling effects of sweating (McCutcheon & Geor, 2014; McEwan, Jenkinson, Elder & Bovell, 2006). Given the challenges of working in such a tropical climate, parasitic infections that may result in a range of diseases, including anaemia and hypoproteinaemia, will not only limit the working lives of ponies but may indeed be fatal (American Association of Equine Practitioners, 2019; Febriyanti *et al.*, 2019; Nugraha *et al.*, 2018; Nurcahyo, Yowi, Hartati, & Prastowo, 2019).

Although studies have reported on the prevalence of gastrointestinal and vector-borne parasitic diseases, such as Piroplasmosis and Trypanosomiasis, of equids in many provinces of Indonesia (Febriyanti *et al.*, 2019; Nugraha *et al.*, 2018; Nurcahyo *et al.*, 2019), to our knowledge there have been no reported studies of such in NTB.

The aim of this study was therefore to investigate key health and welfare indices of working ponies in NTB including assessment of body condition score (BCS), clinical parameters and recovery following exercise,

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[1] Veterinary aid clinics sponsored by charitable organisations Animal Aid Abroad, Gili Eco Trust and Horses of Gili. From April 2020 the Covid-19 pandemic restrictions interrupted the regularity of these clinics which are anticipated to resume in mid to late 2022.

haematological indices and direct examinations for intestinal and blood-borne parasites. We anticipate that this pilot study will provide the basis for more intensive and targeted future studies.

MATERIAL AND METHODS

STUDY DETAILS

Clinical records from a total of 454 clinical examinations of ponies were analysed following three veterinary aid clinics conducted on four islands within NTB province, Gili Trawangan, Gili Meno, Gili Air and the south-eastern region of the larger Lombok Island (Gerung and Mataram). Data from biannual clinics conducted from November 2018 to November 2019 were analysed.

The average maximum daily temperature during the periods that the clinics were conducted, in April-May and in November, ranged from 30-31.5°C and humidity from 76% to 80% respectively (Anonymous, 2019).

Clinical data, observations, and general history were recorded on a veterinary record proforma for each pony presented to the clinic (refer to Supplementary Item A1). The proforma was informed by previously designed and validated assessment tools, including the Animal Welfare Indicators (AWIN) (Dalla Costa *et al.*, 2017) and the Standardised Equine-Based Welfare Assessment Tool (SEBWAT) (Sommerville *et al.*, 2018). The form was modified, with practicality and safety in mind, to suit field examinations in a population consisting primarily of stallions i.e. excluding many parameters that may have put the veterinarians safety at risk. Practicalities relating to these examinations, such as time constraints and veterinary resources, were also considered in the prioritisation of fields recorded. At the commencement of each clinic, veterinary examiners were trained and supervised by a senior veterinarian (CME) and a veterinary epidemiologist (PLH) on conduct of the examinations and recording of information on the proforma. It was estimated that between 55-85% of the annual working pony population from the Gili Islands were represented in each clinic (Delphine Robb, Gili Eco Trust, personal communication). Ponies were examined in central locations on the islands. Following arrival at the clinic, ponies were offered water and shade. Details of the pony and owner were recorded prior to clinical examination, including horse sex, horse age (years), and estimated height and weight. Only ponies ≥1 year of age were included in analysis of clinical assessments (n=4 foals excluded). Clinical assessment of parameters was performed in most cases at least 30 minutes after exercise was completed. Temperature (°C), heart rate (HR, beats per minute), respiratory rate (RR, breaths per minute), mucous membranes (normal, pale, dark); skin tent and capillary refill time (CRT) (1 second, 2 seconds, ≥3 seconds); and body condition score (BCS 1 poor; 5 very fat) were recorded. Abnormal clinical signs were classified as respiratory rate (tachypnoea >30 breaths/minute), heart rate (tachycardia >44 beats/minute) and rectal temperatures (hyperthermia > 38.5°C) (Flethøj *et al.*, 2014; Hubert & Beadle, 2002; Jenkinson, *et al.*, 2007; MacKay, *et al.*, 2015; Mayhew & Ferguson, 1987). For entire male ponies, a subjective visual assessment of the degree of scrotal relaxation in terms of pendulousness was also recorded (+ scrotum contracted close to the body; ++ scrotum in normal position; +++ scrotum fully descended). Given the potential risk of close contact with the inguinal region, no objective measurements were taken. Evidence of sweating, or absence of, was recorded either by noting moisture or staining of skin during the April/May and November 2019 clinics only**.** The absence of sweating (observed anhidrosis) was only recorded when at least one clinical parameter was elevated (RR, HR, and or pyrexia) that would be considered stimulatory to a sweat response in horses.

FAECAL EGG COUNTS

Fresh convenience faecal samples were collected from ponies following defecation during the November 2019 clinic (n=19). Samples were processed and analysed in the field by faecal floatation using a saturated sodium chloride floatation solution, following a modification of the protocol in the AAEP Parasite Control Guidelines (American Association of Equine Practitioners, 2019). Four grams of faeces were suspended in 4 mL of water and mixed with 52 mL of floatation solution. Two chambers of the McMaster slide were counted (1 mL total) giving a detection limit (multiplication factor) of 15 eggs per gram (EPG). Samples were left to settle in the McMaster slide for 5-10 minutes prior to microscopic examination.

BLOOD SAMPLING

Blood samples were collected and analysed from ponies in the November 2018 clinic (n=18). Venepuncture was performed from the jugular vein of ponies. Five ml whole blood was collected into an EDTA vacutainer tube (BD Vacutainer® EDTA Tubes, https://www.bd.com/en-us), refrigerated at 4° C and transported to the Faculty of Veterinary Medicine University of Gadja Mada Jogjakarta Indonesia (UGM). Haematological analysis was performed using an Abaxis HM5© (https://www.abaxis.com). In addition, blood smears were prepared and stained using the Giemsa stain for microscopic examination. Blood smears were examined for protozoal parasites including *Trypanosoma evansi* and the haemoprotozoans *Theileria equi*, and *Babesia caballi.*

DATA ANALYSIS

Mean and standard deviations of physiological parameters prevalence, and Wilson score 95% confidence intervals (95% CI) of ponies with abnormal clinical signs are presented. To determine whether BCS had changed across the three clinics we used the Kruskal-Wallis rank test. To determine differences in the proportion of abnormal clinical signs over successive clinics we used logistic regression. Analyses were conducted in Stata/SE Version 15.1 (College Station, TX: StataCorp LLC). Summary descriptive statistics (percentage detected, mean, standard deviation, median, range) were generated for the faecal egg count and haematological results using Microsoft Excel (Redmond, WA: Microsoft Corporation).

RESULTS AND DISCUSSION

CLINICAL DATA

Records for 143 ponies were available from the 26-30 November 2018 clinic, 145 ponies from the 22 April to 3 May 2019 clinic, and 166 ponies from the 25-29 November 2019 clinic were available. Descriptive characteristics of ponies and results of clinical evaluations are outlined in Table 1. The mean age of ponies was 7.6 years (±4.7; range 1 to 25 years). The majority of ponies were stallions (365/447, 81.7%), with 50 mares (11.2%) and 32 (7.2%) geldings.

The mean BCS across all clinics was 2.89 (±0.49; range 1.5, 4.5; n=362), with no significant difference between clinics (P= 0.297). Eighty-five percent of ponies (95% CI 80.50, 87.92; 307/363) assessed presented with tachypnoea, 37.24% presented with tachycardia (95% CI 32.78, 41.92; 159/427), 14.80% (95% CI 11.10, 19.46; 41/277) recorded rectal temperatures considered hyperthermic (>38.5°C), and 38.0% showed no evidence of sweating after exercise (95% CI 32.21, 44.16; 95/250) with neither moisture nor evidence of evaporation and staining of skin in typical areas under friction. Ponies examined at the April/May 2019 clinic were more likely to be considered hyperthermic (P= 0.009) and/or present with tachycardia (P<0.001), whereas ponies examined in the November 2019 clinic were more likely to present with tachypnoea (P=0.001), when compared to the other clinics.

Thirteen percent of ponies (43/321) presented with pale or dark mucous membranes; 3% with ≥3 seconds of skin tenting (12/404) or capillary refill time (11/400), with such parameters often indicative of cardiovascular compromise including dehydration or illness, however, it is difficult to quantify without more precise analysis than in the field (Pritchard, Barr, & Whay, 2006). Forty-nine percent (95% CI 42.42, 54.79; 120/247) of stallions examined were considered to have extensive scrotal relaxation (+++) consistent with cremaster and dartos muscle relaxation.

Faecal samples from 19 adult working male ponies from the November 2019 clinic were collected and analysed (refer Supplementary Table S1). Strongyle eggs were detected in eight of the nineteen ponies (40%). The mean count was 111 EPG (median 0; range 0-540 EPG). Only two ponies recorded counts over 100 EPG. One faecal sample revealed the presence of an adult pin worm (*Oxyuris Equi*). Other parasitic diseases evident clinically included ocular habronemiasis in four ponies and onchocerciasis was suspected with acute pruritic dermatitis over the dorsum and ventral midlines in two ponies.

Blood samples were collected from 18 ponies during the November 2018 clinic. No protozoal organisms typical of piroplasmosis or Trypanosomiasis were detected following Giemsa staining and microscopic examination of whole blood (Woods & Walker, 1996). Haematological evaluation was performed at the UGM veterinary laboratory using their reference ranges (Southwood, 2013). Most ponies recorded haemograms within normal limits (refer Table S2). Seven ponies recorded marginally low haemoglobin levels (between 10-11 g/dl normal >11g/d), with the Packed Cell Volume (PCV) of two ponies below 32% (Laboratory reference minimum), one of these recording a low red blood cell count (4.8 $\times10^6$ cells/μL, Laboratory reference range 6.2–10.2 $\times10^6$ cells/μL) consistent with anaemia. Mild hypereosinophilia was detected in two ponies (1.03 and 1.23 (Laboratory reference range 0-1.0 x10^3/μL). One pony recorded a leucocytosis of 12.8 (Laboratory reference range 4.9 –12.5 ×103 cells/μL). Neutropaenia was evident in a single pony (Neutrophils 0.69 $\times10^3$ cells/μL (Laboratory reference range 2.2–9. 5 $\times10^3$ cells/μL).

Although parasitic skin diseases were clinically evident in several ponies, an obvious hypereosinophilia was detected in only two ponies. Such a finding in horses is generally associated with either parasitism or atopic-type responses (McKenzie, 2013), however there were no clinical abnormalities detected in these two ponies.

No haemoprotozoan parasites were obvious upon direct examination of stained blood smears Although relatively insensitive compared to specific serological tests (Nugraha *et al.*, 2018; Nurcahyo *et al.*, 2019), these examinations provided some insight into the health status of the ponies. Given the health consequences of Piroplasmosis and Trypanosomiasis and their presence on other islands of Indonesia continued surveillance is indicated for these vector-borne diseases.

The general health, parasite burden, and body condition of the ponies surveyed in NTB were considered consistent with good health and condition. In addition, the ponies were also considered free of disease as a population. However, abnormal clinical parameters were recorded in a high proportion of ponies, tachypnoea being evident in the majority, as was reported in a previous study of this population (Pinsky *et al.*, 2019). This was likely due to the ambient temperatures >30°C and humidity >75% resulting in challenging thermoregulatory conditions for otherwise clinically healthy working ponies. As well as this, it was usual practice for the majority of ponies to exercise in most instances pulling a cart for greater than 10 minutes trotting prior to arrival at a central location away from most stables. A minority of ponies presented without pulling a cart however it was extremely rare

Table 1. Details for 454 clinical examinations of ponies during clinics conducted on four islands of Nusa Tenggara Barat province relating to clinical abnormalities that could be associated with thermoregulatory stress (n: number of ponies), 2018 to 2019.

	November 2018 N=143*			April 2019 N=145			November 2019 N=166		
	n	Mean (sd)	Range	n	Mean (sd)	Range	n	Mean (sd)	Range
Age (years)	109	8.21 (4.80)	1, 25	126	7.58 (4.79)	1, 25	136	7.10 (4.51)	1, 20
Estimated height (hh)	131	12.2 (1.1)	9, 15	105	13.0 (1.1)	10.2, 15	112	12.2 (0.8)	10.5, 15
Estimated weight (kg)	123	318.86 (27.73)	180, 450	56	256.07 (58.55)	120, 450	92	246.68 (49.28)	150, 400
BCS (1-5)	129	2.88 (0.36)	1.5, 4	119	2.86 (0.59)	1.5, 4.5	114	2.93 (0.50)	2, 4
Temperature (°C)	97	37.70 (0.64)	36.6, 39.9	102	38.15 (0.87)	36.7, 40.7	78	37.80 (0.61)	36, 39
Heart rate (beats/min)	132	41.17 (7.78)	28, 76	139	48.40 (10.14)	28, 82	156	44.21 (8.80)	28, 80
Respiratory rate (bpm)	89	48.46 (23.01)	16, 148	121	54.63 (29.82)	10, 160	153	55.77 (17.75)	15, 116
	n	%	95% CI	n	%	95% CI	n	%	95% CI
Sex									
Male entire	111	81.0	–	116	80.6	–	138	83.1	–
Male gelding	10	7.3	–	10	6.9	–	12	7.2	–
Female	16	11.7	–	18	12.5	–	16	9.6	–
Skin tents (sec)									
1	77	61.6	–	117	86.7	–	104	72.2	–
1 to 2	19	15.2	–	1	0.7	–	–	–	–
2	25	20.0	–	14	10.4	–	35	24.3	–
≥3	4	3.2	–	3	2.2	–	5	3.5	–
CRT (sec)									
1	55	43.0	–	105	79.0	–	67	48.2	–
1-2	29	22.7	–	–	–	–	–	–	–
2	43	33.6	–	25	18.87	–	65	46.8	–
≥3	1	0.8	–	3	2.34	–	7	5.0	–
Mucous membranes									
Normal (pink)	74	91.4	–	108	90.8	–	96	79.3	–
Pale pink	7	8.6	–	10	8.4	–	25	20.7	–
Dark pink	0	0.0	–	1	0.8	–	0	0.0	–
Scrotal relaxation‡									
+	6	12.2	–	9	9.4	–	12	11.8	–
++	19	38.8	–	40	41.7	–	41	40.2	–
+++	24	49.0	–	47	49.0	–	49	48.0	–
Abnormal clinical signs	n	%	95% CI	n	%	95% CI	n	%	95% CI
Temperature (>38.5°C)	8	8.25	4.24, 15.44	24	23.53	16.35, 32.63	9	11.54	6.19, 20.50
Tachycardia (>44 beats/min)	27	20.45	14.46, 28.12	79	56.83	48.53, 64.78	53	33.97	27.01, 41.71
Tachypnoea (>30bpm)	69	77.53	67.82, 84.96	95	78.51	70.38, 84.89	143	93.46	88.39, 96.41
No observed sweat	–	–	–	54	42.18	33.98, 50.85	41	33.61	25.84, 42.38
Obvious scrotal relaxation (+++) ‡	24	48.98	35.58, 62.53	47	48.96	39.19, 58.80	49	48.04	38.59, 57.63

*Excludes n=4 foals (<1 years of age); † n=1 excluded, classified as pigmented; ‡ assessed in entire males only. Skin Tents -time to restored position after manual pinching/tenting, CRT = Capillary refill time noted on mucous membrane after digital pressure applied.

for drivers or riders to walk these ponies to the clinics. Under such ambient conditions recovery time to resting respiration rate after exercise may also be increased (Correa *et al.*, 1966; Hubert & Beadle, 2002) above what is considered normal clinically normal in equids (Hodgson, 2014a, 2014b; Lekeux *et al.*, 2014) These signs are a reflection of the greater oxygen requirement increasing respiratory rate and blood flow to maximise cooling processes (McCutcheon & Geor, 2014). Further evidence of thermoregulatory stress was confirmed in almost half of the stallions surveyed with obvious scrotal relaxation and distension indicative of cremaster and dartos muscle relaxation, mechanisms to dissipate heat (McCutcheon & Geor, 2014). Of concern was a lack of obvious sweat response in more than one-third of ponies following exercising in tropical conditions, although definitive diagnosis of non-sweating (anhidrosis) would require intradermal stimulation, this condition is often described as an observation following heat distress in horses lacking an associated sweat response (Hubert & Beadle, 2002; Jenkinson *et al.*, 2007; Mayhew & Ferguson, 1987; McCutcheon & Geor, 2014; McEwan *et al.*, 2006). Recognition and further surveillance and investigation into anhidrosis are warranted given the welfare consequences of this condition in these ponies.

Haematological assessment of ponies provided a valuable addition to clinical examination particularly relating to erythron and leucon values with few ponies recording marked abnormalities. Although one pony was considered anaemic, these results provided a degree of assurance that the ponies in general were not suffering from obvious inflammatory conditions indicated by leucocytosis and or anaemia.

Although blood samples were collected at least 30 minutes after exercise, the erythron parameters in some ponies may have been elevated due to splenic contraction associated with exercise and possible hypovolaemia (McKenzie, 2013). Ideally further clinical pathological measurements may have assisted estimation of any circulatory compromise, that was suspected in ponies with delayed skin tent and CRT. Unfortunately, logistical issues in the field compromised more expansive clinicopathological testing.

Faecal floatation with sodium chloride allows for the detection of strongyle, ascarid, and cestode infections (Dryden, *et al.*, 2005; Norris *et al.*, 2018), although the technique is not considered sensitive enough to reliably detect cestode infections (Tomczuk *et al.*, 2014). The lack of ascarid eggs was not surprising given that all ponies were mature, a stage where immune responses are likely to suppress ascarid infections in horses (American Association of Equine Practitioners, 2019). The correlation between faecal worm egg counts and intestinal worm burdens remains unclear hence the difficulty in attributing clinical significance (American Association of Equine Practitioners, 2019; Saeed *et al.*, 2010; Saeed *et al.*, 2019). Current parasite control guidelines categorise horses shedding levels of 200 EPG or lower as low-level shedding, requiring minimal anthelmintic treatments and less likely to contaminate pasture (American Association of Equine Practitioners, 2019; Kaplan & Nielsen, 2010). Seven of eight (88%) with strongyle shedding ponies recorded low FECs. The pony that recorded the highest FEC of 540 EPG was able to graze, as it was kept on a small paddock with other horses. The high prevalence of low FECs in this study could be due to several factors. These include the likelihood that the limited grazing available on the Gili Islands has minimised exposure to larval pickup. The majority of ponies are stabled and fed rice flour, cut grass as well as pelleted feed in elevated troughs (Pinsky *et al.*, 2019). Although there are some zoonotic parasitic infections of horses, those studied in this work were not considered to be of major One Health significance compared to other relevant issues in this space to about working ponies of NTB.

This study provides some initial guidance regarding the ongoing welfare of working ponies in the NTB province. We found that the general health and body condition of our study population was considered acceptable, and the FEC was considered low in the majority of samples, this was reflected clinically in the lack of obvious signs of intestinal parasitism. Of concern was that many ponies recorded prolonged recoveries from exercise, with tachypnoea being evident in most. Given the general health of the ponies, most of which had been accustomed to working, it is possible that they were suffering from a degree of thermoregulatory stress. We recommend that authorities allow provision for shade and cooling of these ponies and ensure regular breaks during hot periods of the day to minimise physiological stress associate with exercise in tropical conditions. Though most of our findings are to be expected, they have not been well documented for working equids in this region. Future studies should be conducted with more objective health and welfare measures and on larger sample sizes, where practical, to confirm these findings. Methods to identify ponies over subsequent clinics more efficiently may also aid in understanding improvements within individuals as well as at the population level. Investigation of associations between prior exercise undertaken, tachypnoea, tachycardia, hyperthermia, and other important welfare issues such as underlying pain due to musculoskeletal injuries or respiratory problems, should also be explored to understand the full welfare burden on these ponies. Though COVID-19 restrictions disrupted the biannual clinical surveys, continuance of these in the future, and improved forms of data capture will aid in monitoring of the health and welfare of these ponies over time. Not only will these assist initiatives to improve welfare, it may provide important One Health impacts that assists ponies, their owners and carers and may also assist the ongoing support of tourism in the NTB province and beyond.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing interests.

ETHICS STATEMENT

Although retrospective in nature any clinical samples taken were as part of health assessments were performed under the licence and ethics permit of University of Gadja Mada, Jogjakarta, Indonesia. Owners or their representative's informed consent was obtained prior to taking blood and faecal samples and conducting veterinary examinations.

AUTHOR CONTRIBUTIONS

Conceptualisation and methodology, PLH and CME; data analysis, AD and JV under supervision of PLH; laboratory analysis, DA JV and AJ; data collation, JV, SM, AD and PLH; original draft preparation, AD, PLH and CME; review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

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