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Clinical abnormalities in working donkeys and their associations with behaviour

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ABSTRACT

Introductions: Working donkeys are at risk of developing multiple, acute and chronic health problems. The ability to recognise and assess pain in donkeys associated with these health problems is important for people responsible for their care and treatment, including owners and veterinary or animal health workers.

Aims and objectives: The aims of this study were firstly to quantify the prevalence of a range of clinical abnormalities within a sample of working donkeys; and secondly to find out whether these abnormalities were associated with potential behavioural indicators of pain.

Materials and methods: One hundred and thirty-three entire male adult working donkeys were observed for ten minutes before and after a one-hour rest period. Using an ethogram developed and refined in associated studies, posture and event behaviours were recorded by a single observer. The health of each donkey was then assessed by a veterinarian for specific clinical abnormalities.

Results: Working donkeys have a high prevalence of clinical abnormalities and a number of behaviours are associated with these. Significant associations were found between observed behaviours and systemic, ocular and limb-related clinical abnormalities. Cumulative clinical scores for limb-related problems were associated with a higher frequency of leg trembling, knuckling of the forelimb, leg-lifting and weight-shifting behaviours (all $R \geq 0.4$; $P<0.001$) and with a lower frequency of weight-bearing evenly on all four feet ($R=-0.458$; $P<0.001$).

Conclusions: The specific behaviour changes associated with clinical abnormalities identified in this study, together with general changes in demeanour identified in related studies, may be useful in assessing the presence and severity of pain in working donkeys and their response to medical and palliative interventions.

INTRODUCTION

There are an estimated 43.5 million donkeys worldwide (FAOSTAT 2013), the majority of which are working animals which support people living in the world's poorest communities, by transporting a wide range of goods and materials by pack or cart. As a consequence of their work requirements, living conditions and handling, many donkeys develop multiple, acute and chronic health problems (Pritchard and others 2005). The ability to recognise and assess pain in donkeys associated with these health problems is important for people responsible for their care and treatment, including owners and veterinary or animal health workers.

Behavioural indicators of limb pain (Price and others 2003), foot pain (Wright 1993), eye pain (Hendrix 2005), back pain (Wolf 2002), abdominal pain (Muir and Robertson 1985) and dental pain (Lane 1994) have been recorded for the horse, and similar behaviours have been observed and quantified in donkeys working in developing countries (Ashley and others 2006, Regan and others 2014, 2015). However, published information describing the behaviour of domestic donkeys in clinical contexts is limited (Ashley and others 2005). Clinical research relating to pain and pain management in donkeys has been undertaken primarily using healthy animals to investigate pharmacokinetic profiles of drugs (Matthews and others 1994, 2001), responses to anaesthetic regimens (Matthews and others 1992, 2002) and behavioural considerations when dealing with mammoth asses in a veterinary environment (Taylor and Matthews 1998). A review of the scientific literature on equine pain (Ashley and others 2005) found that behaviours described in horses were not reported for the donkey. This may be for several reasons: donkeys may not demonstrate clear source-specific behavioural indicators of pain, but exhibit a non-specific general change in demeanour which cannot be easily linked to particular pathologies

(Taylor and Matthews 1998), donkeys studied previously may not have been in sufficiently acute pain to demonstrate the more obvious pain behaviours; or the many anecdotal reports of their stoical behaviour may be hindering further study of clinical behaviour, in the belief that it is unnecessary.

The aims of this study were first to quantify the prevalence of a range of clinical abnormalities within a sample of working donkeys; and secondly to find out whether these abnormalities were associated with observable behaviours which may prove useful as indicators of pain.

MATERIALS AND METHODS

Ethics statement

The study was carried out in Lahore, Pakistan, under ethical approval from the University of Bristol Faculty of Medical and Veterinary Sciences Ethics Committee (Investigation number UB/05/017) and was compliant with Pakistan law regarding ethical use of animals in science.

Donkeys

The high prevalence of clinical conditions within this population, with donkeys typically exhibiting more than one health problem, meant that it was not possible to perform accurate sample size calculations or to recruit control groups of donkeys free from pain for each of the conditions explored in this study. To prevent sex differences influencing study findings only entire male donkeys were chosen as subjects (Regan and others 2014).

In total 133 entire male adult working donkeys were recruited outside a wayside clinic in Lahore, Pakistan run by The Brooke. All donkeys were used to transport goods by cart or pack and were suffering from health issues including poor body condition, wounds, ocular conditions, dental abnormalities and lameness. Exclusion criteria included acute abdominal or respiratory distress, severe medical conditions such as tetanus or shock, inability to walk with reasonable encouragement and stereotypical behaviour patterns. Donkeys entering or receiving treatment at the clinic were not recruited. Selection of subjects was not random given the opportunistic sampling strategy; whoever was passing the clinic at the appropriate time, had an eligible donkey and gave their consent to participate in the study was recruited rather than a targeted strategy being adopted.

On arrival each donkey was placed in a holding area and offered 1 kg chopped maize forage, in order to distinguish pain-related behaviour from hunger. Water was available to all donkeys ad libitum throughout the study.

Experimental protocol

From the holding area, each donkey was moved to a 3 m×3 m sand-floored observation pen placed 8 m away from a busy road, replicating the examination area normally used by the clinic's veterinary staff. In the observation pen the donkey was tied loosely to a ground ring for five minutes, then a single handler (JA) entered the pen, untied the lead rope, held it 1.5 m from the donkey's head and remained still for another five minutes to habituate the donkey to the handler's presence. The handler did not interact or make eye contact with the donkey, except to encourage it to stand up at the start of the study period if it was lying down; following this the donkey was allowed to stand, lie or roll as it chose. The observer (FHR) then observed the behaviour of the donkey for 10 minutes, following which the donkey was tied again and the handler left the pen. This procedure (five minutes habituation to handler; 10 minutes observation) was repeated after one hour to test for a potential effect of rest on the behaviours expressed. Another donkey was continuously present in an adjoining pen so that the donkey was not socially isolated between and during the two observation sessions.

Behavioural observation

A single observer (FHR), seated 3.5 metres away from the front of the pen, used a previously developed ethogram (Table 1; Regan and others 2014) to record behaviour. The behaviours recorded fell into two types, Postural and Event behaviours, and were recorded in different ways. Anatomical components of posture (ear orientation, eyelid position (open/closed), muzzle tension, head carriage, limb placement and tail position) were recorded each minute over the 10 minute observation period using instantaneous (scan) sampling, giving us 10 observations of each measure for each observation session. In contrast all occurrences of the predefined event behaviours (eg, rolling, walking, eating, rubbing; see Table 1 for full list) were counted over the same 10-minute period, giving us one record for each behaviour for each 10 minute observation session.

Clinical examination

Immediately after the second behavioural observation period, each donkey was examined by a single veterinarian (JG) blinded to the previous behavioural observations. Clinical abnormalities were recorded as present or absent (1 or 0) with the exception of body condition score which was rated on a five-point scale and response to palpation of the back, tail and forelimbs which was recorded on a four-point scale as described by Broster and others 2009 (see Table 2). Heart rate, respiratory rate and rectal temperature were recorded as continuous variables. The clinical examination check sheet is available as online supplementary material.

Statistical analysis

The prevalence of each clinical problem was calculated (percentage of donkeys with each abnormality out of the 133 donkeys sampled). For each postural behaviour

TABLE 1: Ethogram of behaviours observed in domestic (including working) donkeys at rest

Category	Behaviour	Description
Pen position	Back of pen	Head and forelimbs in back half of pen (irrespective of orientation)
	Front of pen	Head and forelimbs in front half of pen (irrespective of orientation)
Postural behaviours		
Lying down	Lying sternally	Lying down on sternum, legs folded underneath body frame
	Lying laterally	Lying on side with legs outstretched, head and neck may be in contact with ground
Standing	Standing on four feet	Weight-bearing on all four limbs with no preferred loading
	Standing on three feet	Weight-bearing on three limbs with a hindlimb resting
	Pointing	Placing a foot in a forwards position outside of the main body frame (minimum one hoof length) with reduced weight-bearing
	Knuckled	Forwards bend of one or both forelimbs with the knee bent in front of the placed foot or fetlock joint
Ear position	Forwards	Both ears facing forwards with ear cups fully visible when facing the donkey head on
	Sideways	Both ears facing sideways with ear cup orientated approximately 90 degrees laterally from forwards-facing position
	Backwards	Both ears facing backwards with ear cups visible when standing behind the donkey
	Combinations	Each ear in a different orientation, for example, one facing forwards, one facing backwards
Ear level	Down	Tip of ear level with or below level of base of ear, drooping downwards in any orientation
	Up	Tip of ear above base of ear, in any orientation
Head carriage	High	Poll higher than top of withers
	Level	Poll level with top of withers
	Low	Base of ears below top of withers
	Very low	Nose contact with ground
Head direction	No turn	Straight head carriage, no turning of head towards self, object or stimuli
	Turn to belly	Head is turned towards or makes contact with either side of abdomen
	Turn to flank	Head is turned towards or makes contact with either flank
	Turn to limb/foot	Head is turned towards or makes contact with either forelimb/hindlimb/foot
	Turn to look	Head is orientated away from a straight position with attention drawn towards environmental stimuli and not directed towards a body part/region
Tail position	Relaxed	Tail held in a relaxed position, hanging freely in a vertical line from its body base
	Lifted out	Tail held in a fixed position, sticking out more than 45 degrees from the vertical line
	Tucked	Tail held tightly against the rump in a fixed position, with tip of tail tucked between hindlegs
	Swishing	Tail moves swiftly from its base in a side-to-side flicking manner around the hindquarters
Event behaviours		
Body	Rolling	From lying down laterally or sternally, vigorous rolling and wriggling movement of whole body over onto back
	Transition up/down	From lying down laterally or sternally to standing, or vice versa
Feet	Walking	Forwards, backwards or sideways movement of limbs to a new position
	Pawing	Repetitive lifting and backwards dragging or scraping of pointed hoof
	Leg lifting	Temporary lifting of any limb from the ground with hesitant replacement near to its original position, often repetitive
	Weight shifting	Weight temporarily offloaded from a fore or hindlimb onto the remaining three limbs, accompanied by subtle body rock
	Foot stamping	Sudden lifting and forceful replacement of any limb in its original position
Oral	Eating	Prehension of food into the mouth, with repetitive chewing and swallowing
	Drinking	Muzzle touches water source followed by at least one visible swallow
	Sniffing	Movement of muzzle towards object, ground or body, followed by inhalation and movement of nostrils
	Flehmen response	Upper lip curls back to expose gums with incisors meeting, head tips back and rapidly points muzzle upwards
	Licking and chewing	Repetitive licking and chewing motion in the absence of any food in the mouth
	Yawning	Mouth opens wide, eyes close, head rises, tips back and shakes, lower jaw grinds and closes

Continued

TABLE 1: Continued

Category	Behaviour	Description
	Biting	Grasps object, self or another donkey in open mouth and bites or chews with single or repeated jaw closures
Head	Head shaking	Vigorous rotational shake of head and neck resulting in ears flapping against sides
	Head tossing	Rapid up and down movements of neck with successive nods of head
Vocal	Snorting	Quick forced exhalation of air through nostrils making an audible noise
	Braying	Series of short duration, loud inhalations, followed by a prolonged noisy exhalation
	Sighing	Prolonged deep inhalation with a noticeable rise of the body, followed by a short burst of expiration, then gradual release of air
	Groaning	Similar to a sigh, but accompanied by a monotone prolonged vocalisation and often head is turned towards body part
	Coughing	Distinctive short duration exhalation from the lungs, deep and often moist in sound, often accompanied by a body heave
Maintenance	Self grooming	Repeated grooming movement of mouth and incisors directed at own body parts
	Rubbing	Moving a body part against another (eg, eye rubbing on foreleg) or repeatedly moving a body part back and forth against any object
	Stretching	Head tucks into neck, mouth opens slightly, eyes often close, whole body contracts and rises up noticeably with inhalation
	Dozing	State of body stillness with eyes closed, head lowered and standing on all four feet, or standing on three feet and resting a leg
Eliminative	Defaecating	Elimination of faeces
	Urinating	Elimination of urine
Play	Playing	Lifting up and tipping/holding objects with mouth, rearing, broncing, bucking or attempted interaction

the number of times it was recorded in each observation session was calculated giving each postural behaviour a score from 0 to 10 for each of the two observation sessions. The total number of occurrences of each event behaviour was calculated for each session; this was a continuous measure with each event behaviour scoring ≥0. Data were not normally distributed, so non-parametric Wilcoxon's signed rank tests for matched pairs were used to investigate differences in behaviour between the first and second observation sessions for each donkey, to test for any effect of rest. As no significant differences were found the remainder of the analyses use the behaviour data obtained in the first observation session.

Relationships between the behaviours observed were explored using Spearman's rank correlations. Due to the high prevalence of clinical conditions within this population and collinearity of many of the behavioural measures (see results) the data were not suitable for multivariate analysis. Instead Mann-Whitney U tests for independent samples were used to explore behavioural differences between donkeys with and without each clinical condition where the prevalence of the condition in the 133 donkeys sampled was ≤80 per cent and ≥10 per cent (see Table 2 for the prevalence of each clinical abnormality). Cumulative scores were calculated for eye abnormalities and limb abnormalities by totalling the scores (1 present, 0 absent) that each donkey received for each grouping. The cumulative number of wounds was also calculated by totalling the wounds present in each body area to create a total number of wounds for each donkey.

Spearman's rank correlations were used to test for associations between the frequency of behaviours and cumulative number of wounds, problems affecting eyes or problems affecting limbs as well as heart rate, respiratory rate and rectal temperature.

As there was a high risk of Type I errors (finding false positives) through the multiple tests conducted, Holm's sequentially rejective multiple test procedure (Holm 1979) was used post hoc for all tests. The adjusted P value denoted by the procedure was $P \leq 0.001$ and only variables meeting this adjusted value and thereby retaining their significance were reported as such. SPSS V.21.0 (SPSS, Illinois, USA) was used for all analyses.

RESULTS

The majority of donkeys recruited were 5–15 years of age (62 per cent), with 27 per cent <5 years old and 11 per cent aged 16 years or older. None of the donkeys had a body condition score greater than 3 (medium); 12 per cent were scored 1 (very thin), 72 per cent scored 2 (thin) and 16 per cent scored 3 (medium) on a scale of 1–5 (1=very thin, 5=very fat).

All of the donkeys recruited had multiple conditions of varying severity and at different stages of natural progression. Table 2 presents the prevalence of clinical abnormalities in the study population. All of the donkeys sampled had sole defects and swollen joints, while 98 per cent had observable lameness (score 2). A restricted range of movement was seen in the forelimbs of 98 per cent of the sample, swollen tendons in 97 per

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TABLE 2: Clinical observations in 133 entire adult male working donkeys, expressed as a prevalence within the study group and as median+IQR for continuous physiological data, some palpation scores and cumulative abnormality scores

Observation	% donkeys	Observation	% donkeys	Observation	% donkeys
*Demeanour**		*Nostrils/muzzle/dental*		Tail—response to palpation median (IQR)†	1 (1–1)
Restless	3	Slit nostrils	13	0=no response to palpation	11
Bright and alert	46	Flared or fixed nostrils	12	1=responds to light touch using one finger	68
Apathetic	49	Discharge (uni/bilateral)	72	2=medium touch using middle three fingers	13
Dull and depressed	2	Nostril asymmetry	89	3=firm touch using palm or whole hand	8
		Lip lesions	90	Tail base—swellings	67
Systemic		Hypersalivation	1	Tail—mutilation/amputation	16
Abnormal lung sounds	97	Incisor overbite	80		
Abnormal borborygmi	88	Damaged or broken incisors	3	*Limbs*	
Capillary refill time >2 seconds	14	Sharp molar edges/hooks	86	Forelimb—response to palpation median (IQR)†	1 (1–1)
Abnormal mucous membrane colour	17	Pocketing feed in cheeks	6	0=no response to palpation	1
Heart rate: median (IQR)	45 (42–50)			1=responds to light touch using one finger	76
Respiratory rate: median (IQR)	20 (16–25)	*Hooves*		2=medium touch using middle three fingers	16
Rectal temperature: median (IQR)	38 (37–38)	Bounding digital pulse	61	3=firm touch using palm or whole hand	7
		Coronary band swollen	86	Forelimb—restricted range of movement	98
Wounds‡		Split or damaged hoof wall	90	Swollen joint(s) —any limb(s)	100
Head	45	Sole defects	100	Swollen tendon(s) —any limb(s)	97
Ear	2	Response to palpation of coronary band	99	Forelimb(s) affected more severely than hindlimb(s)	31
Neck	4			Hindlimb(s) affected more severely than forelimb(s)	69
Breast	60	*Body Frame*			
Withers	26	Neck—response to palpation median (IQR)†	1 (1–2)	*Lameness§*	
Ribs	18	0=no response to palpation	4	0=no gait abnormality	0
Belly	74	1=responds to light touch using one finger	52	1=uneven gait	2
Hindquarters	14	2=medium touch using middle three fingers	25	2=observable lameness	98
Tail	23	3=firm touch using palm or whole hand	19		
Limb	40	Neck—swellings	65	*Evidence of¶:*	
		Neck—restricted range of movement	93	Firing	1
Eyes				Hobbling	87
Discharge (uni/bilateral)	60	Back—response to palpation median (IQR)†	1 (1–2)	Tethering	5
Wet eyelashes	29	0=no response to palpation	4	Blistering	1
Steep eye lash angle	74	1=responds to light touch using one finger	70		

Continued

TABLE 2: Continued

Observation	% donkeys	Observation	% donkeys	Observation	% donkeys
Red/swollen conjunctivae	10	2=medium touch using middle three fingers	17	*Cumulative scores***:	
Corneal abnormality	84	3=firm touch using palm or whole hand	9	Number of wounds: median (IQR)	4 (2–5)
Globe abnormality	17	Back—swellings	92	Number of problems affecting eyes: median (IQR)	3 (2–4)
Absent menace response	12	Back—restricted range of movement	92	Number of problems affecting limbs: median (IQR)	10 (8–11)
Ears		Hindquarters/pelvis asymmetrical	60		
Slit or broken	4	Hindquarters—muscle atrophy	55		
Ear mite damage	4				

*Demeanour scored based on Pritchard and others (2005)
‡Wounds scored according to Pritchard and others (2005), where severity scores (0–3) relate to the area and depth of tissue damage. Hair loss, wounds or scars resulting from blistering, firing and hobbling were recorded separately
†Response to palpation from Broster and others (2009)
§Evaluated over 20 strides at walk
¶From Pritchard and others (2005)
**Cumulative scores calculated by totalling the scores (1 present, 0 absent) each donkey received for abnormalities that fell into each grouping (wounds, eyes, limbs)
IQR, interquartile range

cent and abnormal lung sounds were detected in 97 per cent of the donkeys.

Effect of rest on behaviour

There were no significant differences in the behaviour observed in the first and second observation sessions. For example, lying down had a median of 0 in both observation sessions (Z=45.0, P=0.637), walking had a median of 1 in both sessions (Z=2048.0, P=0.707) and weight-shifting had a median of 4 in the first observation session and 5 in the second session, however, this difference was not statistically significant (Z=3671.5, P=0.096).

The remainder of the following analyses uses the behaviour data obtained in the first observation session.

Associations between the behaviours observed

In total 45 different postural and event behaviours were observed during the study period; associations between some of these behaviours are presented in Table 3. Fifty-nine correlations between behaviours were greater than or equal to R=0.4 indicating a moderate correlation. Walking was strongly positively correlated with sniffing (R=0.871). There was also a strong positive correlation between having a knuckled forelimb and trembling leg.

Associations between clinical and behavioural findings

Associations between systemic health problems and behaviour

Weak negative correlations were found between respiratory rate and both ears being held sideways (R=−0.295; P<0.001) and eyes being closed (R=−0.343; P<0.001), indicating that the higher the respiratory rate the fewer times ears held sideways and eyes closed were seen across the 10 observation points. A positive correlation was found between respiratory rate and swishing the tail (R=0.291; P=0.001). Rectal temperature was positively correlated with tail swishing (R=0.312; P<0.001) and negatively correlated with tucking the tail between the hindlegs (R=−0.341; P<0.001).

Abnormal mucous membrane colour was associated with a higher frequency of lying down (Mann-Whitney U 1475.0; abnormal mucous membrane colour present median=0 (interquartile range (IQR) 0–0, min=0, max=9), abnormal mucous membrane colour absent median=0 (IQR 0–0, min=0, max=2); P<0.001) and a lower frequency of holding both ears forward in an alert/vigilant position (Mann-Whitney U 630.0; abnormal mucous membrane colour present median=0 (IQR 0–0), abnormal mucous membrane colour absent median=2 (IQR 0–4); P<0.001).

No significant associations were identified between heart rate, capillary refill time >2 seconds, abnormal borborygmi, and any of the behaviours observed.

Associations between ocular problems, limb problems, hindquarter abnormalities and behaviour

Significant associations between ocular problems, limb problems, hindquarter abnormalities and behaviour are presented in Table 4. Hindquarter asymmetry had the

TABLE 3: Significantly (P<0.001) correlated behaviour with R≥0.4 in 133 entire adult male working donkeys

Positive correlations

Correlated behaviours	Correlation coefficient (R)
Weight-bearing evenly	
Walking	0.412
Sniffing	0.454
Knuckled forelimb—trembling leg	0.629
Both ears forward	
Head held alert (poll above withers)	0.484
Walking	0.455
Sniffing	0.514
Turn to look at environmental stimuli	0.458
Both ears sideways	
Eyes closed	0.434
Head held low	0.443
Head held alert (poll above withers)	
Walking	0.466
Sniffing	0.456
Licking/chewing	0.442
Turn to look at environmental stimuli	0.492
Head very low	
Rolling	0.424
Walking	0.577
Sniffing	0.629
Turning to flank	0.420
Snorting	0.405
Tail out—walking	0.416
Trembling leg	
Lifting leg	0.417
Shifting weight between limbs	0.460
Rolling—turning to flank	0.451
Walking	
Sniffing	0.871
Flehmen response	0.400
Licking/chewing	0.551
Turning to look at environmental stimuli	0.543
Snorting	0.463
Touching handler	0.483
Sniffing	
Flehmen response	0.405
Licking/chewing	0.567
Turning to flank	0.421
Turn to look at environmental stimuli	0.596
Snorting	0.484
Touching handler	0.483
Flehmen response—snort	0.535
Licking/chewing—turning to look at environmental stimuli	0.450
Turning to limb—self grooming	0.632
Self grooming—turning to belly	0.434
Lifting leg—shifting weight between limbs	0.431
Rubbing body—rubbing eye	0.771

Negative correlations

Correlated behaviours	Correlation coefficient (R)
Weight-bearing evenly	
Knuckled forelimb	−0.439
Pointed limb	−0.536
Trembling leg	−0.492
Lifting leg	−0.416
Shifting weight between limbs	−0.435
Both ears forward	
Both ears sideways	−0.535
Eyes closed	−0.589
Both ears sideways	
Both ears back	−0.675
Head held alert (poll above withers)	−0.634
Eyes closed	
Head held alert (poll above withers)	−0.578
Walking	−0.468
Sniffing	−0.477
Turning to look at environmental stimuli	−0.559
Head held alert (poll above withers)	
Head level with withers	−0.474
Head lower than withers	−0.614
Relaxed tail—swishing tail	−0.758
Muzzle normal—muzzle droopy	−0.865

greatest number of significantly associated behaviours, and these encompassed a range of different behaviours including ear position, head carriage, weight distribution and oral behaviours.

TABLE 4: Clinical abnormalities significantly associated with behaviour in 133 entire adult male working donkeys

Clinical abnormality	Behavioural indicator	Clinical abnormality present Median* (IQR)	Clinical abnormality absent Median* (IQR)	Mann-Whitney U test statistic	Level of significance P value
Ocular problems					
Eye discharge	Closed eyes	8 (3–10)	2 (2–8)	2873.0	<0.001
Steep eyelash angle	Head level with withers	3 (1–7)	0 (0–3.25)	2360.0	<0.001
Limb problems					
Bounding digital pulse	Turn towards belly	0 (0–0)	0 (0–1)	1648.5	<0.001
	Lifting limb	0 (0–1.5)	0 (0–0)	2404.0	0.001
Forelimbs more severely affected than hindlimbs	Knuckled forelimb	1 (0–4.5)	0 (0–0)	2555.0	<0.001
	Lifting limb	0 (0–3)	0 (0–0)	2443.0	<0.001
Hindlimbs more severely affected than forelimbs	Knuckled forelimb	0 (0–0)	1 (0–4.5)	1217.0	<0.001
	Lifting limb	0 (0–0)	0 (0–3)	1328.0	<0.001
Problems with the hindquarters					
Hindquarter asymmetry	Ears held to the side	5 (2–9)	3 (1–5)	2879.0	<0.001
	Weight shifting between limbs	6.5 (3–12)	3 (1–4.5)	3104.0	<0.001
	Ears held forwards	1 (0–3)	3 (1–5)	1353.5	<0.001
	Walking	0 (0–2.75)	5 (0–11)	1235.0	<0.001
	Sniffing	0 (0–3)	3 (0–5)	1295.0	<0.001
	Chewing/biting†	0 (0–0) (min=0,max=0)	0 (0–0) (min=0, max=8)	1800.0	<0.001
	Turn to look at environmental stimuli	2 (0–4)	5 (2–8.5)	1146.0	<0.001
	Lifting limb	0 (0–1.75)	0 (0–0)	2624.0	0.001
	Limbs weight-bearing evenly	2 (0–7)	7 (1.5–10)	1419.0	0.001
	Very low head	0 (0–0)	0 (0–1)	1530.0	0.001
	Licking/chewing	2 (1–3)	3 (2–5)	1395.0	0.001
Muscular atrophy of hindquarters	Chewing/biting†	0 (0–0) (min=0, max=0)	0 (0–0) (min=0, max=8)	1898.0	0.001

*Median (IQR) portrays the median number of times the behaviour was observed out of a maximum of 10 times for postural behaviours and median number of times the behaviour was observed over the whole observation session for event behaviours
†Min and max provided to show direction of effect not seen in the IQR
IQR, interquartile range

Behaviours associated with cumulative clinical abnormalities

Table 5 presents the behaviours associated with cumulative scores for wounds and limb conditions. Weak negative correlations were found between cumulative wound scores and walking, sniffing and snorting. Moderate positive correlations were found between cumulative limb abnormalities score and trembling legs, weight shifting, knuckling of the forelimb and lifting the legs. Cumulative limb abnormalities score was moderately negatively correlated with weight-bearing evenly on all four limbs, that is, the higher the cumulative score of limb abnormalities, the less often the donkey was observed bearing it's weight evening on all four limbs. There were no significant associations between any behaviour and cumulative eye abnormalities score.

DISCUSSION

The aims of this study were to quantify the prevalence of clinical abnormalities within a sample of entire male working donkeys and to find out whether these abnormalities were associated with potential behavioural indicators of pain. There was a high prevalence of clinical abnormalities within the working donkeys sampled and many of the observed behaviours were significantly correlated with

TABLE 5: Behaviours significantly associated with accumulation of wounds and clinical limb abnormalities in 133 entire adult male working donkeys

Accumulation of clinical abnormalities	Behaviours observed more frequently (positive R) or less frequently (negative R) in animals with higher cumulative clinical score	Correlation coefficient (R)	Significance (P)
Cumulative wound score			
	Walking	−0.322	<0.001
	Sniffing	−0.292	0.001
	Snorting	−0.286	0.001
Cumulative limb score			
	Trembling leg	0.636	<0.001
	Bear weight evenly on all four feet	−0.458	<0.001
	Shifting weight between limbs	0.457	<0.001
	Knuckled forelimb	0.419	<0.001
	Lifting leg	0.407	<0.001
	Sniffing	−0.387	<0.001
	Very low head carriage	−0.384	<0.001
	Walking	−0.350	<0.001
	Turn to look at environmental stimuli	−0.348	<0.001
	Relaxed tail	0.338	<0.001
	Swishing tail	−0.311	<0.001
	Licking/chewing	−0.291	0.001
	Both ears forward	−0.282	0.001

each other, as well as being associated with specific clinical abnormalities, and with cumulative limb or wound scores.

No significant differences in behaviour were found between the first and second observation periods, indicating that an hour-long rest period does not affect the behavioural expression of working donkeys. This has important methodological implications for future studies on this population, as it suggests that there may be no benefit of waiting to observe behaviour until after a rest period and equally that any delay in observations will not significantly affect the behaviour observed. However, it should be noted that the behavioural observations used in this study were relatively broad in nature, and more detailed observations may have detected an effect of rest on subsequent behaviour.

Auscultation of the lungs and abdomen demonstrated a high prevalence of abnormal lung sounds, which may be attributable to the animals' polluted working environment. This raises concerns for donkey health, work capacity and longevity. No other data on respiratory disorders in animals living or working in comparable environments were found. However, there may be parallels drawn with the health of the human population in this region, and greater understanding of the effects of environmental pollutants on animals may help to inform or support studies on human subjects (Reif 2011).

Although the findings of this study suggest that ocular abnormalities are prevalent within the working donkey population, research on ocular pain in animals is limited and almost non-existent for donkeys. Anecdotal comments suggest that domestic donkeys show a muted pain response compared with horses (Inglis 1997). Behavioural indicators of eye pain in horses include blepharospasm and frequent blinking (Hendrix 2005), while even a small downward change in eyelash angle may indicate ocular pain and discomfort (Plummer 2005). In this study, donkeys with eye discharge were more frequently observed with their eyes closed and those with a steep eyelash angle were more often seen with their head held with their poll level with the withers. Some donkeys did not display a menace response, although this abnormality had no consistent behavioural association. While this may indicate a defect in the visual nervous pathway and suggest partial or complete blindness in one or both eyes, it has also been stated that depressed or stoic animals may show a reduced menace response (Lunn and Mayhew 1989) and this may provide an alternative explanation for the lack of response seen in the donkeys sampled.

Abnormal borborygmi were found in 88 per cent of the donkeys sampled. Many factors could contribute to abdominal abnormalities in working donkeys including poor quality and irregular feed, little or no long-stem forage, poor hydration status, dental malfunction, systemic health problems and ingestion of foreign objects such as plastic bags. Classic behaviours associated with acute equine abdominal pain, such as kicking at the abdomen (Muir and Robertson 1985), rolling (Crane 2002), flank watching (Pritchett and others 2003) and stretching (Jochle 1989) were used as exclusion criteria for this study; donkeys showing these signs were sent directly for treatment. Although not part of the study, donkeys were seen entering the field clinic with visible indicators of acute abdominal pain, including rolling, groaning, stretching and sweating, despite dullness and depression being commonly reported as the only behaviours indicating abdominal pain in the domestic donkey (Whitehead and others 1991, Matthews and others 1997, Duffield and others 2002).

Working donkeys suffering from chronic and/or episodic acute pain as a result of musculoskeletal problems are likely to show reduced work performance, with consequences for their owners' livelihoods. Feral asses rarely gallop (Moehlman 1998), yet working donkeys are frequently required to trot and gallop on uneven and hard ground (including roads) from a young age, while pulling carts or carrying pack-loads. This is likely to account for the high prevalence of limb problems and lameness observed in the present study donkeys (98 per cent), comparable with that of working donkeys assessed in previous surveys (Pritchard and others 2005, Reix and others 2014).

Some limb abnormalities were found in all donkeys so could not be analysed for behavioural differences between affected and unaffected animals. These included swollen joints, swollen tendons, a restricted range of movement in forelimbs and split or damaged hoof walls. There is an abundance of literature regarding limb and foot pain in the domestic horse, but comparable evidence for the donkey is lacking. Anecdotal descriptions suggest an absence of the postural changes seen in the horse: such as no obvious alteration in the stance of donkeys with laminitis (Trawford and Crane 1995); often the only behavioural changes were an increase in lying time and a reduction in movement. Stiffness and difficulty rising are seen in donkeys suffering from arthritis (Whitehead and others 1991). Source-specific behavioural indicators of limb and foot pain in equids, including weight-shifting, leg lifting and abnormal weight distribution (Ashley and others 2005), were observed in donkeys in the present study and these were associated with the presence of clinical abnormalities such as asymmetrical hindquarters/pelvis, a bounding digital pulse and donkeys whose forelimbs were more severely affected than their hindlimbs. These behavioural indicators of limb pain have immediate application to the improvement of pain recognition, assessment and management in the donkey. The frequency of limb-related behaviours also correlated positively with the cumulative number of clinical problems affecting the limbs, indicating their usefulness as part of a tool to monitor the severity of limb pain and response to treatment strategies.

This study benefited from being carried out entirely in the field, replicating clinicians' opportunities for observing behavioural signs of pain and relating them to a clinical examination. As such, the findings of this study have significant practical application for future studies on working donkey populations. The study environment accurately represented the environment in which behaviours are normally performed and animals were observed for a realistic duration (10 minutes). The behavioural repertoire was not diminished by the busy environment, if anything the donkeys appeared more relaxed and expressive than in previous studies when they had been moved to quieter locations for observation (Regan and others 2014, 2015). Behaviours were selected to be directly relevant for assessing pain and response to treatment, as described in equine literature and investigated in previous two studies by the same authors (Regan and others 2014, 2015).

However, practical field studies bring their own limitations: it was not possible to recruit equal numbers of donkeys with each clinical abnormality, or control groups, due to the complex clinical picture presented by the majority of working donkeys. Similarly, due to the scarcity of information on the prevalence of clinical abnormalities in working donkeys, sample size calculations could not be performed. While the study sample was opportunistic by necessity, the study design and analysis were intended to reduce variability as much as possible by using only male animals, habituating them to the observation area and handler and accounting for any potential effect of rest on behaviour. Behaviours that are strongly influenced by time of day (Ashley and others 2006, Regan and others 2014) were also excluded. Although the presence of a handler lightly restraining the donkey will have some impact on behaviour, observations were made in the presence of a handler to replicate an authentic situation when an animal is presented to the clinic for examination. Despite these precautions it is not known how representative the findings of this study are of other populations of working donkeys and this should be given consideration when interpreting the results of this study.

The high prevalence of clinical abnormalities and the majority of donkeys being presented with multiple conditions also made the exploration of significant associations between behaviour and clinical conditions more complex. While post hoc tests were used to control for the multiple tests conducted, it should be understood that the findings of this study are not as clear-cut as those from studies exploring such relationships in subjects with only one condition and a control group. Some of the correlations identified were relatively weak ($R<0.4$) despite the high level of statistical significance required to retain significance after the post hoc test. These relationships would benefit from further exploration, ideally using donkeys affected by only the condition of interest and an appropriate control group. The interrelationships between the behaviours observed also warrant further investigation and may help to refine behavioural observations and analysis in future studies.

The assessment of the donkeys' health was designed to document the presence or absence of abnormalities and not as a diagnostic clinical examination evaluating the severity of every abnormality found. The logical progression from this study would be to incorporate an evaluation of clinical severity into the assessment protocol, alongside an indication of the impact this degree of abnormality may have on the donkey in terms of pain and suffering. The findings of this study have immediate practical application however, and can be used to augment existing clinical practice at roadside treatment sites and equine field clinics by including more focused behavioural observations in the clinical examination process. These will inform treatment decisions and enable better assessment of response to analgesia and palliative management of pain in working donkeys.

CONCLUSION

Working donkeys with a range of clinical abnormalities show both general and specific behavioural indicators of pain. Some behaviours were expressed more frequently in association with cumulative clinical abnormalities. These behaviours may have the potential to be used as a tool for assessing pain severity and response to medical and palliative interventions. As such these behaviours potentially have great value in enabling owners to monitor the welfare of their donkeys, to determine when to solicit treatment and to evaluate the efficacy of any treatment undertaken.

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