Histological evidence of superficial inflammation is associated with lower recurrence of equine sarcoids following surgical removal: A follow-up study of 106 tumours in 64 horses

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ABSTRACT

Although the equine sarcoid is the most common skin neoplasm in domesticated horses, histopathological characteristics have not previously been evaluated for association with recurrence. The aim of this retrospective cohort study was to investigate clinical and histopathological features of excised equine sarcoids and to evaluate their association with recurrence at the original surgical site and at new sites. Clinical records and excisional biopsies from 106 equine sarcoids from 64 horses referred to Leahurst Equine Hospital, University of Liverpool, between March 2010 and February 2015 were retrieved. Biopsies were re-evaluated histologically. Clinical data were obtained from hospital records, and owner-reported follow-up data were obtained by telephone questionnaire. Associations between clinical and histopathological features of sarcoids and their recurrence at the surgical site were determined using uni- and multivariable mixed effects logistic regression. Recurrence of sarcoids at the surgical site occurred in 30 horses (46.9%). Sarcoids developed at a distant site in 21 horses (32.8%). In the final mixed effects logistic regression model, only superficial inflammation was associated with reduced odds of recurrence at the surgical site (adjusted odds ratio, 0.32; 95% confidence intervals, 0.10–0.96; $P = 0.04$). This suggests that the inflammatory process may play a role in protecting horses against the recurrence of sarcoids.

Introduction

Sarcoids are the most frequently submitted equine neoplasm for histopathological analysis (Valentine, 2006; Schaffer et al., 2013; Knowles et al., 2016). They are difficult to treat, with recurrence rates varying from 20% to 80% (Knottenbelt and Kelly, 2000; Martens et al., 2001; Compston et al., 2016). Some risk factors for sarcoid re-growth have been identified, including size, repeated treatment (Martens et al., 2001), and periocular location (Knottenbelt and Kelly, 2000). Although proliferative markers, such as Ki67 and CD47 have been evaluated for their presence in sarcoid tissue (Martens et al., 2000; Nixon et al., 2005; Bogaert et al., 2007; Bogaert et al., 2011; Caston et al., 2016), their association with recurrence has not been determined. Similarly, the identification of Bovine Papilloma Virus (BPV) DNA

within sarcoid tissue, which has largely been investigated for the differentiation of sarcoids from other fibrocellular tumours, such as peripheral nerve sheath tumours (PNSTs) (Martens et al., 2000; Bogaert et al., 2005; Bogaert et al., 2008), has not been investigated for its association with sarcoid recurrence.

Diagnosis of sarcoids is generally based upon characteristic clinical appearance prior to surgical excision and histopathological confirmation (Taylor and Haldorson, 2013; Haspeslagh et al., 2018). Sarcoids are histopathologically characterised by plump, haphazardly arranged fibroblastic cells, mild/moderate atypia, and variable number of mitotic figures, often on the low end (Martens et al., 2001). These neoplastic cells form fibrocellular masses that are often whorled in appearance. Epidermal acanthosis, hyperkeratosis, and hyperplasia with rete pegs into the dermal fibroblastic tissue are also common features

(Knottenbelt and Kelly, 2000; Martens et al., 2001), together with epidermal ulceration.

Histopathology of an excised sarcoid has similarities to that of schwannomas, exuberant granulation tissue, or other mesenchymal neoplasms (Taylor and Haldorson, 2013; Wobeser, 2017). Unfortunately, there is often marked heterogeneity in expression of immunohistochemical markers such as S100 across sarcoids, fibrosarcomas, PNSTs, and other sarcomas (Epperson and Castleman, 2017). Moreover, histological features detectable on routinely stained haematoxylin and eosin (HE) samples have not been evaluated as prognostic indicators for sarcoid recurrence.

The aim of this retrospective cohort study was to determine the recurrence prevalence, and the clinical and histological factors associated with recurrence of equine sarcoids treated by laser/sharp excision.

Materials and methods

Inclusion criteria and clinical data

Medical records at Leahurst Equine Hospital, University of Liverpool, were reviewed for horses presented with at least one sarcoid for treatment between 1 March 2010 and 1 January 2015, for which a histological diagnosis of equine sarcoid had been reached and there was formalin-fixed, paraffin-embedded (FFPE) tissue retained at the Section of Veterinary Pathology, University of Liverpool. All horses were referred from surrounding equine veterinary practices for treatment. Horses were excluded when there was a lack of follow-up information, or the sample was from a recurrent lesion that had previously been treated surgically, or when the histological sample could not be matched with a specific location and treatment (Fig. 1).

Data recorded from hospital records included age (at time of sarcoid diagnosis), sex, and breed. Date of surgical biopsy, anatomical location of sarcoids, sarcoid type (i.e., nodular, verrucose, fibroblastic, or occult) (Knottenbelt, 2005), any record of previous medical treatment of the sarcoid and the described method of surgical removal of the sarcoid sampled, were also obtained. In general, a combination of diode laser/sharp resection was used for surgical removal of the sarcoids and a margin of at least 1 cm of visibly and palpably normal tissue around the sarcoid was obtained.

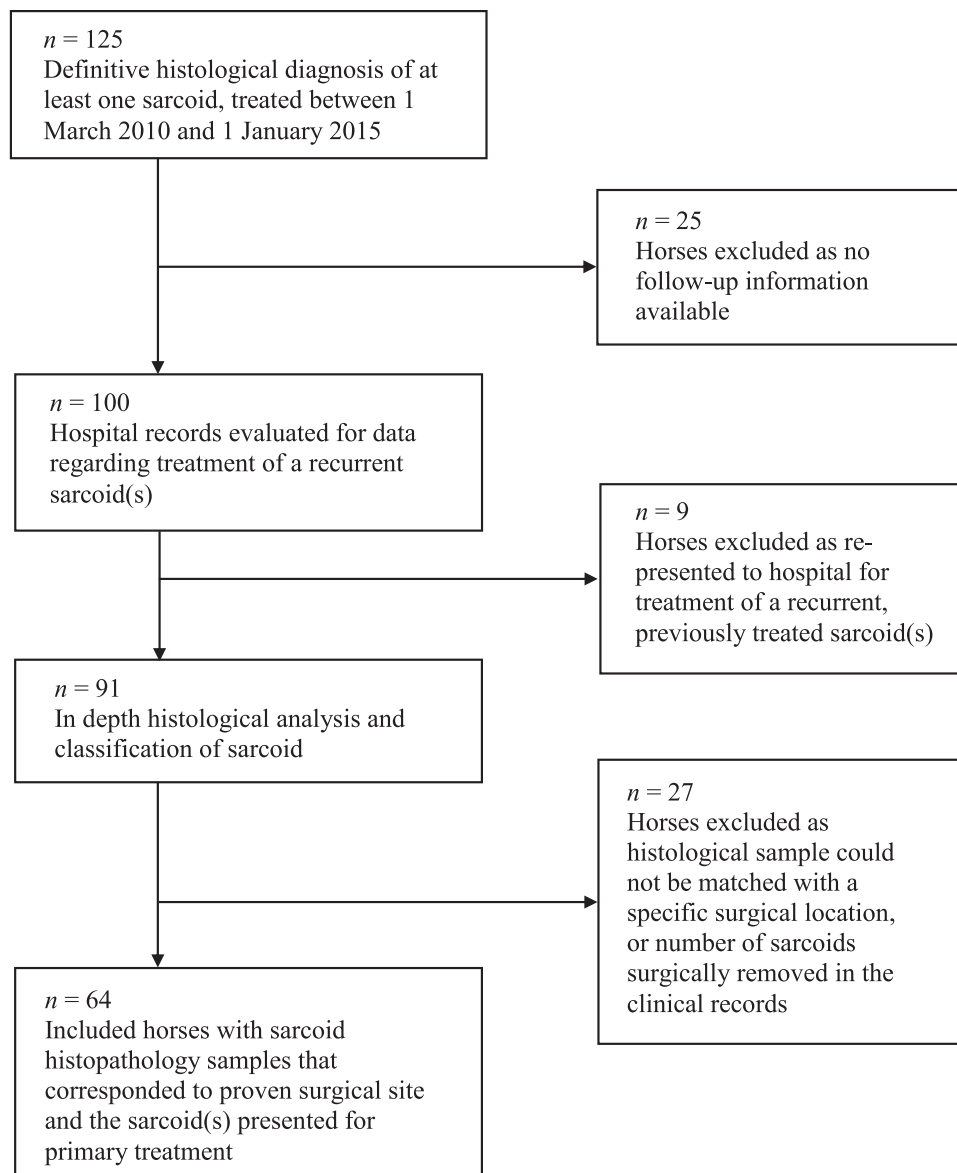Sarcoid location was divided into four anatomical sites: (1) limb

Fig. 1. Flow diagram showing the progression of selection of horses through the inclusion and exclusion criteria.

(including the hoof to outer thigh/shoulder); (2) axilla and ventral abdomen; (3) head and neck; and (4) inguinal, sheath, mammary, and medial thigh.

Follow-up data were obtained by telephone questionnaire between 2 January 2019 and 1 April 2019, (Supplementary Table S1), consisting of closed and open questions examining whether (and if so when) the horse had died or had been sold following surgery and histological sampling. All respondents answered the call on the first attempt. Owners were asked whether any new sarcoids had developed, their anatomical location and type (if known), and the date the new sarcoid was noticed. Follow-up was considered unavailable if owners could not be contacted by telephone after three attempts at different times of the day. Based on follow-up data, horses were categorised into four groups: (1) no sarcoid recurrence at the original site or occurrence at a new location; (2) recurrence only at the original surgical site; (3) occurrence of a sarcoid at a new location only; and (4) recurrence of the original sarcoid and occurrence in a new location.

The primary outcome variable was defined as any sarcoid regrowth at the original surgical site. A secondary outcome variable was defined as any new sarcoid growth at a distant site from the original surgical site.

Ethical approval was granted by the University of Liverpool Committee on Research Ethics (Approval number, VREC271a; Approval date, 20 November 2014).

Histopathological analysis

Following surgical excision and complete fixation in 10% neutral buffered formalin solution, samples were paraffin-embedded, sliced into 4 μm sections, mounted on glass slides, and stained with hematoxylin (Hematoxylin, TCS Biosciences Ltd) and eosin (Eosin, TCS Biosciences Ltd) (HE).

Two pathologists performed histopathological analysis of the biopsy samples using light microscopy. The first pathologist (L.R.) confirmed the diagnosis of equine sarcoid histologically. The second pathologist (A. R.) assessed each neoplasm according to the presence of the following diagnostic features: (1) a dermal fibroblastic proliferation haphazardly arranged in short streams, interlacing bundles and whorls embedded in collagenous and poorly vascular stroma (Ragland et al., 1970; Goodrich et al., 1998; Knottenbelt et al., 2015); (2) perpendicular arrangements along the epidermal basement membrane (picket-fence pattern); (3) ovoid to spindled neoplastic cells, with indistinct cell borders, scant pale eosinophilic fibrillary cytoplasm and single slender elongate to plump spindled nuclei with variable anisokaryosis (Goodrich et al., 1998); and (4) epidermal hyperplasia confirming a 'biphasic nature' (Ragland et al., 1970; Goodrich et al., 1998; Knottenbelt et al., 2015).

Stained samples were subsequently evaluated using a semi-quantitative scoring system, using the following 13 criteria: mitotic index, nuclear pleomorphism, indication of a biphasic nature, completeness of excision, demarcation, pseudocapsulation, growth pattern, neoplasm location, presence of inflammation, type, and location of inflammation, and the presence of ulceration or furunculosis. Descriptions of the diagnostic criteria are given in Table 1.

Statistical analysis

Data were analysed using SPSS Statistics 27 (IBM). Categorical data were presented as percentages. Based on graphical methods, skewness, kurtosis and results of Kolmogorov-Smirnov and Shapiro-Wilk tests, no continuous variables were normally distributed and consequently were presented as medians with interquartile ranges (IQR).

To examine the prognostic relationship between anatomic site of sarcoid at the time of diagnosis and the risk of sarcoid recurrence at the surgical site, survival analysis was performed. The length of time to sarcoid recurrence (days at risk) was defined as the time from date of surgical biopsy (entry date) to date of sarcoid recurrence at the original surgical site. Observations were right censored if there was no

Table 1

Description of the histopathological criteria used for assessment of each equine sarcoid.

Histopathological Criterion	Description
a) Mitotic Index	Mitotic figures in ten non-overlapping high-power fields (Microscopic 400x; ocular FN: 22x; objective 40x/0.65). Atypical mitoses defined as non-bipolar or non-symmetrical figures (multipolar, asymmetrical, ring, dispersed, and lag-type mitoses) were included (Van Leeuwen et al., 1995). Peculiar structures (pyknotic or karyorrhectic nuclei or apoptotic bodies) were excluded (Matsuda et al., 2016).
b) Nuclear Pleomorphism	Variation in nuclear size, shape, and staining (Rashid and Haque, 2011), affecting > 10% of neoplastic cells.
c) Biphasic Nature	Epidermal hyperplasia with rete pegs extending deep into the subepidermal proliferation of neoplastic fibroblasts (Goodrich et al., 1998) across > 10% of the central/paracentral regions of the neoplasm.
d) Excision Completeness	Neoplastic cells not observed at the surgical margins.
e) Demarcation	Neoplastic borders clearly distinguishable from adjacent non-neoplastic dermal tissue.
f) Pseudocapsulation	Fibrotic capsule at the neoplasm borders: a) Complete b) Incomplete c) Absent
g) Growth Pattern	a) Expansile - outward centrifugal homogeneous growth with compression of adjacent dermal adnexa and stroma tissue. b) Infiltrative - insidious multifocal penetration of the dermis with the neoplastic invasion between mildly displaced normal tissue.
h) Neoplasm Location	a) Superficial - dermo-epidermal junction to sebaceous glands. b) Middle - between sebaceous glands and hair bulb base. c) Deep - below the hair follicle with underlying subcutis and deeper fascia/muscle layers included.
d) Inflammation	> 10 inflammatory cells in discrete clusters, cords, or sheets identified in direct contact and/or infiltrating the neoplasm.
e) Inflammation Type	Lymphocytic, neutrophilic, histiocytic, and eosinophilic, with presence of > 10 cells of any subtype in discrete clusters, cords, or sheets. Neoplasm could have more than one inflammatory cell type.
f) Inflammation Location	a) Superficial - within the top third horizontal layer of the neoplasm. b) Middle - within the middle third horizontal layer of the neoplasm. c) Deep - within the bottom third horizontal layer of the neoplasm. d) Periphery - peripheral 200 μm region from the boundary of the neoplasm. e) Perivascular - any dermal layer but only present around blood vessels within the neoplasm. Location category was not limited to a single classification if multiple locations were identified.
f) Ulceration	Complete epidermal loss directly overlying the neoplasm in association with inflammation.
g) Furunculosis	Inflamed ruptured hair follicles within the neoplasm.

FN, Field Number; μm, micrometre

recurrence of sarcoid at the end of the study (or at the last completed follow-up questionnaire) or if the horse was lost to follow-up at any time during the study (e.g., due to sale or death of the horse). A Kaplan-Meier plot and log rank (Mantel-Cox) test was used to visualise and estimate differences in the probabilities of sarcoid non-recurrence (or 'survival') at the surgical site between the four anatomical sites.

Univariable logistic regression models were used to identify clinical and histological factors associated with the primary outcome of sarcoid recurrence at the surgical site. Because of the potential for variation due to clustering effects (where horses had multiple sarcoids), within-horse clustering was accounted for by incorporation of horse as a random intercept term in all models. Continuous variables (age and mitotic index) were assessed for linearity by conversion to categories and

graphical examination of the shape of the relationship with the outcome, with the categorical variable used where the linear assumption was not met. Prior to univariable analysis, all variables were assessed for correlation using Spearman's rank correlation coefficients.

All variables with a $P < 0.30$ were entered into multivariable mixed effects models, incorporating horse as a random term. Models were built using a stepwise backwards elimination procedure, with concurrent assessment of potential confounding. Variables with $P > 0.05$ were excluded. All excluded variables were then individually re-introduced prior to final model selection. Due to multicollinearity between neoplasm inflammation and inflammation type, separate multivariable mixed effects logistic regression models were built including only one of these variables each, with the final model selected based on the smallest Akaike Information Criterion (AIC).

Associations between relevant explanatory variables and the secondary outcome were assessed using Pearson Chi-squared or Fisher's exact tests, as appropriate. These latter tests were also used, where relevant, to determine associations between selected variables (e.g., method of removal, previous medical treatment, and total number of sarcoids treated surgically) and the primary outcome.

The denominators for all results are 106 for sarcoid-level variables and 64 for horse-level variables, unless otherwise stated, for example where a lack of epidermis precluded complete evaluation for a small number of histopathological variables. Statistical significance was accepted at $P < 0.05$.

Table 2
Descriptive statistics and univariable, mixed effects (multilevel) logistic regression of signalment and clinical factors associated with recurrence at the surgical site in a follow-up study of 64 horses with 106 sarcoids.

Variable	Category n (%)	Sarcoid recurrence at surgical site n (%)	No recurrence at surgical site n (%)	All sarcoids n (%)	Unadjusted odds ratio	95% CI	P
Age	< 5 years 12 (18.8)	10 (47.6)	11 (52.4)	21 (19.8)	2.04	0.51–1.02	0.20
	5–9 years 29 (45.3)	15 (33.3)	30 (66.7)	45 (42.5)	Reference		
	10–14 years 15 (23.4)	7 (28.0)	18 (72.0)	25 (23.6)	0.99	0.26–3.87	
	≥ 15 years 8 (12.5)	11 (73.3)	4 (26.7)	15 (14.2)	5.37	1.00–28.93	
Sex	Male 40 (62.5)	26 (41.9)	36 (58.1)	62 (58.5)	Reference		0.78
	Female 24 (37.5)	17 (38.6)	27 (61.4)	44 (41.5)	0.86	0.29–2.51	
Breed	Native pony breeds and Cobs 18 (28.1)	11 (44.0)	14 (56.0)	25 (23.6)	Reference		0.73
	Thoroughbred/Thoroughbred crosses 11 (17.2)	8 (33.3)	16 (66.7)	24 (22.6)	0.60	0.12–3.11	
	Warmblood/Warmblood crosses 14 (21.9)	15 (51.7%)	14 (48.3)	29 (27.4)	1.38	0.31–6.13	
	Draught breeds/Draught crosses 8 (12.5)	7 (77.8)	2 (22.2)	9 (8.5)	4.86	0.56–41.81	
	Sports Horses 6 (9.4)	0 (0)	10 (100)	10 (9.4)	0.00000003	0.00-	
	Arab 4 (6.3)	0 (0)	4 (100)	4 (3.8)	0.00000003	0.00-	
	Other 3 (4.7)	2 (40.0)	3 (60.0)	5 (4.7)	0.95	0.08–11.99	
Sarcoid location	Inguinal, sheath, mammary, and inner thigh 33 (51.6)	18 (46.2)	21 (53.8)	39 (36.8)	Reference		0.61
	Unknown 2 (3.1)	3 (75.0)	1 (25.0)	4 (3.8)	3.31	0.17–65.91	
	Axilla and ventral abdomen 19 (29.7)	11 (42.3)	15 (57.7)	26 (24.5)	0.93	0.28–3.11	
	Head and neck 24 (37.5)	9 (34.6)	17 (65.4)	26 (24.5)	0.62	0.18–2.09	
	Limb (hoof to outer thigh or shoulder) 10 (15.6)	2 (18.2)	9 (81.8)	11 (10.4)	0.32	0.05–2.13	
Sarcoid type	Nodular 31 (48.4)	17 (39.5)	26 (60.5)	43 (40.6)	Reference		0.90
	Not recorded 27 (42.2)	16 (38.1)	26 (61.9)	42 (39.6)	0.98	0.32–2.98	
	Verrucose 9 (14.1)	5 (45.5)	6 (54.5)	11 (10.4)	0.97	0.19–4.99	
	Fibroblastic 8 (12.5)	5 (62.5)	3 (37.5)	8 (7.5)	2.47	0.39–15.74	
	Occult 2 (3.1)	0 (0)	2 (100)	2 (1.9)	0.0000003	0.00-	
Sarcoid occurrence at a new location	No 43 (67.2)	26 (36.6)	45 (63.4)	71 (67.0)	Reference		0.41
	Yes 21 (32.8)	17 (48.6)	18 (51.4)	35 (33.0)	1.58	0.52–4.73	

95% CI, 95% confidence interval.

Results

Descriptive clinical statistics

Sixty-four horses with 106 sarcoids fulfilled selection criteria (Fig. 1). Sixty-one horses (48.8%) were excluded. Of the 64 included horses, 39 (60.9%) were geldings, 24 (37.5%) were mares, and one (1.6%) was a stallion. Median age was eight years (IQR, 5–11 years). Breeds represented, along with sarcoid characteristics and location, are described in Table 2. Thirty-nine horses had one sarcoid submitted for histological evaluation, twelve horses had two, ten horses had three, and two had four. The maximum number was five sarcoids submitted from one horse, and the overall median was one sarcoid per horse (IQR, 1–2). Of the 25 horses presenting with > 1 sarcoid, 20 had > 1 affected anatomical site and 13 had > 1 different sarcoid types.

The majority of sarcoids were removed using a combination of laser/sharp resection ($n = 95/106$; 89.6%), with a minority using laser resection alone ($n = 10/106$; 9.4%). Method of removal was not associated with recurrence at the surgical site ($P = 0.31$). One periocular sarcoid (0.9%) received brachytherapy following tissue biopsy. This was achieved by using platinum-coated iridium-192 wires, with a radiation dose ranging from 45.0 to 65.0 grey (Gy). Prior medical treatment was reported for 34 sarcoids (32.1%) and was not associated with recurrence at the surgical site ($P = 0.40$).

Median duration of follow-up was 59 months (IQR, 48–76 months). Only four horses had < 6 months of follow-up, of which three had recurrence at the surgical site. Of the 17 horses euthanised, five were euthanised due to sarcoid recurrence.

Based on the owner-reported data, 24 horses (37.5%) had no sarcoid recurrence at the original site or at a new location, 19 horses (29.7%) had sarcoid recurrence only at the original surgical site, 10 horses (15.6%) had occurrence of a sarcoid at a new location only, and 11 horses (17.2%) had recurrence of the original sarcoid and occurrence of another sarcoid in a new location. Overall, 40.6% ($n = 43/106$) of sarcoids that recurred at the surgical site did so a median of 6.8 months (IQR, 3.7–13.6 months) following full surgical excision with margins.

Cumulative probability of sarcoid non-recurrence

The cumulative probability of non-recurrence at the surgical site was higher for sarcoids located on the limb than those located at the other three anatomical sites ($P = 0.05$) (Fig. 2). Kaplan-Meier assumptions were satisfactorily met.
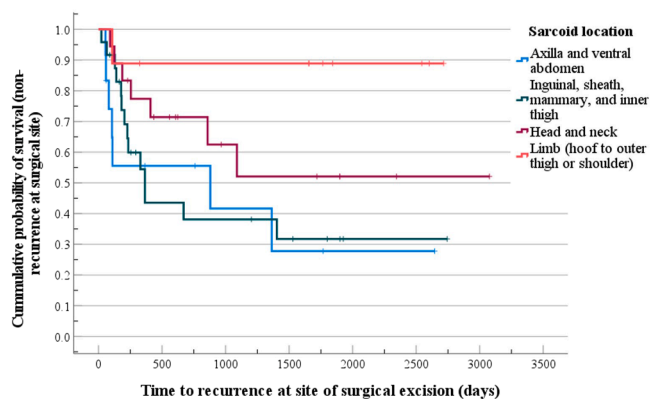

Fig. 2. Kaplan-Meier 'survival' (non-recurrence) curves showing the cumulative probability of non-recurrence of sarcoid at the surgical site over time in 106 sarcoids by anatomical site group. The cumulative probability of recurrence can be determined by subtracting the non-recurrence probability from 1.

Univariable mixed effects logistic regression analysis of clinical characteristics

The total number of sarcoids treated surgically per horse was not associated with sarcoid recurrence at the surgical site ($P = 0.37$) or at a new location ($P = 0.90$). Accounting for within-horse clustering, univariable mixed effects logistic regression analysis found no significant associations between signalment or clinical factors and sarcoid recurrence at the surgical site (Table 2).

Descriptive statistics and univariable mixed effects logistic regression analysis of histopathological characteristics

Descriptive statistics and univariable mixed effects logistic regression analysis of histopathological characteristics are presented in Table 3. Infrequently identified histopathological characteristics are described in Supplementary Table S2, and horse-level descriptive statistics for clinical and histopathological characteristics of the sarcoids are described in Supplementary Table S3.

A minority of tumours were not inflamed (Figs. 3 and 4) while inflammation (Figs. 5 and 6) was detected in 59 tumours (55.7%) in 40 horses (62.5%).

Multivariable mixed effects logistic regression analysis

Significant correlations (all $P < 0.001$) were found between 'neoplasm inflammation' and the 'inflammation types', namely lymphocytic inflammation, neutrophilic inflammation, superficial inflammation, and perivascular inflammation. Based on AIC, the multivariable model including 'superficial inflammation' was determined to have the best fit. Data from all 106 sarcoids (43 that recurred and 63 with no reported recurrence at the surgical site) were included in the final multivariable mixed effects logistic regression model, which correctly classified 91.5% of observations (Table 4). Superficial inflammation (within the top third horizontal layer of the neoplasm), the only variable retained, was associated with reduced odds of recurrence (adjusted odds ratio, 0.32; 95% confidence intervals, 0.10–0.96; Table 4). There was some evidence of within-horse clustering (estimate, 1.36; standard error, 0.73; $P = 0.03$).

Associations with the secondary outcome

For horses with at least one sarcoid recurring at a new location during the follow-up period, median owner-reported time from surgical excision of original sarcoid(s) to observation of the new sarcoid(s) was 11.0 months (IQR, 3.5–32.2 months). There were no statistically significant associations between sarcoid occurrence at a new location and age ($P = 0.39$), breed ($P = 0.20$), presenting with at least two sarcoids at the start of the study ($P = 0.79$), presenting with at least two sarcoid types ($P = 0.52$), presenting with sarcoid(s) in at least two different locations ($P = 0.61$), or having sarcoid recurrence at the surgical site ($P = 0.60$). There was a significant association between sex and sarcoid occurrence at a new location ($P = 0.03$) with 50.0% ($n = 12/24$) of mares having at least one sarcoid reported in a new location compared to 22.5% ($n = 9/40$) of geldings/stallions. Given the small sample size and lack of significant associations demonstrated between clinical features, except for sex, and recurrence of equine sarcoids at distant sites, further logistic regression was not performed.

Discussion

This is the first study to report associations between histopathological features of equine sarcoids and recurrence following surgical excision. The odds of recurrence at the surgical site were significantly reduced in sarcoids that had superficial inflammation, when accounting for within-horse clustering. Clustering at horse level was observed,

Table 3

Descriptive statistics and univariable, mixed effects (multilevel) logistic regression of histopathological factors associated with recurrence at the surgical site in a follow-up study of 64 horses with 106 sarcoids.

Variable	Category	Sarcoid recurrence at surgical site n (%)	No recurrence at surgical site n (%)	All sarcoids n (%)	Unadjusted odds ratio	95% CI	P
Mitotic index (continuous; n = 106 sarcoids)					1.08	0.91–1.28	0.36
Bizarre mitoses	Absent	19 (34.5)	36 (65.5)	55 (51.9)	Reference		
(n = 106 sarcoids)	Present	24 (47.1)	27 (52.9)	51 (48.1)	1.68	0.65–4.33	0.28
Nuclear pleomorphism	Absent	11 (29.7)	26 (70.3)	37 (34.9)	Reference		
(n = 106 sarcoids)	Present	32 (46.4)	37 (53.6)	69 (65.1)	2.16	0.78–5.98	0.14
Indicators of biphasic nature	Absent	24 (36.4)	42 (63.6)	66 (68.8)	Reference		
(n = 96 sarcoids)	Present	14 (46.7)	16 (53.3)	30 (31.3)	1.64	0.58–4.64	0.35
Complete excision	Yes	18 (35.3)	33 (64.7)	51 (49.0)	Reference		
(n = 104 sarcoids)	No	25 (47.2)	28 (52.8)	53 (51.0)	1.76	0.66–4.68	0.26
Sarcoid demarcation	Clear demarcation from adjacent non-neoplastic tissue	12 (29.3)	29 (70.7)	41 (39.4)	Reference		
(n = 104 sarcoids)							0.11
	Poor/no demarcation from adjacent non-neoplastic tissue	30 (47.6)	33 (52.4)	63 (60.6)	2.27	0.83–6.21	
Pseudocapsulation	Non-encapsulated	34 (40.0)	51 (60.0)	85 (81.7)	Reference		
(n = 104 sarcoids)	Partial	3 (50.0)	3 (50.0)	6 (5.8)	1.13	0.14–9.06	0.99
	Complete	5 (38.5)	8 (61.5)	13 (12.5)	0.93	0.22–3.95	
Growth pattern	Expansile	30 (39.5)	46 (60.5)	76 (71.7)	Reference		
(n = 106 sarcoids)	Expansile with multifocal infiltrative features	2 (40.0)	3 (60.0)	5 (5.7)	1.04	0.10–10.97	0.89
	Infiltrative	11 (44.0)	14 (56.0)	25 (23.6)	1.31	0.44–3.92	
Sarcoid location solely within superficial dermal stratum	Yes	19 (37.3)	32 (62.7)	51 (48.6)	Reference		
(n = 105 sarcoids)	No	24 (44.4)	30 (55.6)	54 (51.4)	1.25	0.49–3.16	0.64
Inflammation	Absent	24 (51.1)	23 (48.9)	47 (44.3)	Reference		
(n = 106 sarcoids)	Present	19 (32.2)	40 (67.8)	59 (55.7)	0.43	0.16–1.11	0.08
Lymphocytic inflammation	Absent	29 (46.0)	34 (54.0)	63 (59.4)	Reference		
(n = 106 sarcoids)	Present	14 (32.6)	29 (67.4)	43 (40.6)	0.54	0.20–1.41	0.20
Neutrophilic inflammation	Absent	37 (45.7)	44 (54.3)	81 (76.4)	Reference		
(n = 106 sarcoids)	Present	6 (24.0)	19 (76.0)	25 (23.6)	0.39	0.12–1.25	0.11
Eosinophilic inflammation	Absent	43 (41.2)	61 (58.7)	104 (98.1)	Reference		
(n = 106 sarcoids)							0.99
	Present	0 (0)	2 (100)	2 (1.9)	0.0000007	0.00-	
Superficial inflammation	Absent	36 (47.4)	40 (52.6)	76 (71.7)	Reference		
(n = 106 sarcoids)	Present	7 (23.3)	23 (76.7)	30 (28.3)	0.32	0.10–0.96	0.04
Mid-neoplasm inflammation	Absent	42 (42.0%)	58 (58.0%)	100 (94.3%)	Reference		
(n = 106 sarcoids)							0.37
	Present	1 (16.7%)	5 (83.3%)	6 (5.7%)	0.33	0.03–3.70	
Deep inflammation	Absent	41 (43.6%)	53 (56.4%)	94 (88.7%)	Reference		
(n = 106 sarcoids)							
	Present	2 (16.7%)	10 (83.3%)	12 (11.3%)	0.32	0.05–1.89	0.20
Peripheral inflammation	Absent	40 (40.4%)	59 (59.6%)	99 (93.4%)	Reference		
(n = 106 sarcoids)							0.94
	Present	3 (42.9%)	4 (57.1%)	7 (6.6%)	0.94	0.13–6.55	
Perivascular inflammation	Absent	34 (44.7%)	42 (55.3%)	76 (71.7%)	Reference		
(n = 106 sarcoids)							0.31
	Present	9 (30.0%)	21 (70.0%)	30 (28.3%)	0.58	0.20–1.66	
Ulceration	Absent	31 (44.9%)	38 (55.1%)	69 (74.2%)	Reference		
(n = 93 sarcoids)							0.15
	Present	6 (25.0%)	18 (75.0%)	24 (25.8%)	0.42	0.13–1.38	
Multinucleated giant cells	Absent	37 (40.2%)	55 (59.8%)	92 (87.6%)	Reference		
(n = 105 sarcoids)							0.94
	Present	5 (38.5%)	8 (61.5%)	13 (12.4%)	0.95	0.23–3.88	

95% CI, 95% confidence interval.

suggesting some variation in sarcoid recurrence across individual horses due to factors that were not fitted within the final model. The tumour-specific microenvironment, in particular tumour-associated inflammation, may play a broad although currently unclear role in different types of neoplasms (Allavena et al., 2008; Woldemeskel et al., 2017). Inflammation has been identified as an indicator of improved prognosis in neoplasms such as canine histiocytoma and transmissible venereal tumour in animals, and in subsets of mammary (Matsumoto et al., 2015) and lung tumours (Wang et al., 2013) in humans. It is possible that the presence of inflammation in superficial regions of sarcoids, as found in this study, may stimulate, or re-activate, a reparative process that targets

and counteracts the inciting cause of equine sarcoid, including Bovine papillomavirus (Moore, 2014; Wobeser et al., 2012). As well, investigations regarding tumour-cytotoxic N1-TAN phenotypes suggest an ability of inflammatory cells to kill tumour cells and inhibit their growth (Di Carlo et al., 2001; Kousis et al., 2007). N1-TAN phenotypes additionally activate CD8+ recruitment (Scapini et al., 2000), which may be a mechanism for tumour modulation and adaptive immune responses leading to improved prognosis for some sarcoids.

It is noteworthy that treatments for equine sarcoids, such as the topical chemotherapeutic agents 5-fluorouracil (Stewart et al., 2006; Knottenbelt et al., 2020) and imiquimod (Nogueira et al., 2006;
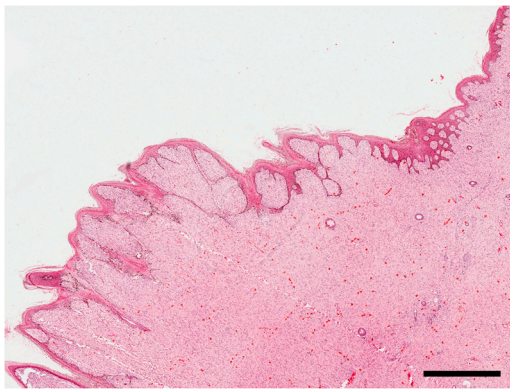
Fig. 3. Horse, skin. Low power magnification of a non-inflamed sarcoid with intact hyperplastic epidermis. (Hematoxylin and eosin; Scale bar: 1 mm).
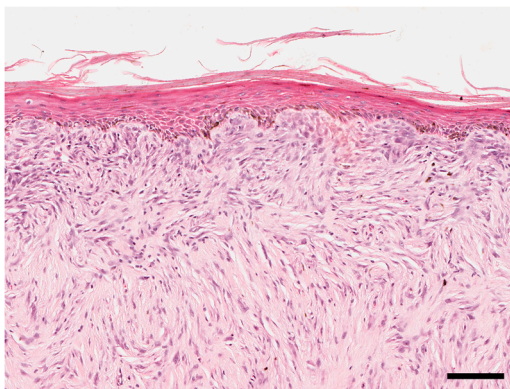

Fig. 4. Horse, skin. high power magnification of a non-inflamed sarcoid. Typical sarcoid features (Hematoxylin and eosin; Scale bar 100 μm).
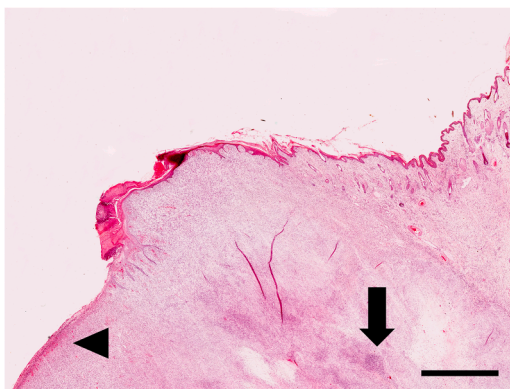

Fig. 5. Horse, skin. Low power magnification of an inflamed sarcoid with evidence of inflammatory infiltrate aggregates (arrow) and ulceration (arrowhead). (Hematoxylin and eosin; Scale bar: 1 mm).

Fig. 6. Horse, skin. High power magnification of an inflamed sarcoid where neutrophils are markedly infiltrating the upper half of the microscopic field. Lower right half of the image shows typical sarcoid features. (Hematoxylin and eosin; Scale bar 100 μm).

Table 4
Final mixed effects (multilevel) logistic regression model of factors associated with recurrence at the surgical site in a follow-up study of 64 horses following surgical excision of 106 sarcoids.

Variable	Category	Adjusted odds ratio	95% CI	P
Superficial inflammation	Absent ($n = 76$)	Reference		0.04
	Present ($n = 30$)	0.32	0.10–0.96	
Within-horse variance, 1.36 (standard error, 0.73)				0.03

95% CI, 95% confidence interval.

Pettersson et al., 2020) probably work by inducing ulceration and active inflammation. Immuno-stimulatory therapies are also used intralesionally for equine sarcoids. These include intralesional BCG vaccine (Martens et al., 2001) and intralesional chemotherapeutic agents such as bleomycin (Souza et al., 2017; Knottenbelt et al., 2020) and cisplatin (Souza et al., 2017). Although the primary action of these drugs is cytotoxic, the subsequent inflammation induced (Cargnoni et al., 2012) may play an additional therapeutic role. The important role inflammation may play in the outcome of sarcoids is also supported by more recent studies that have demonst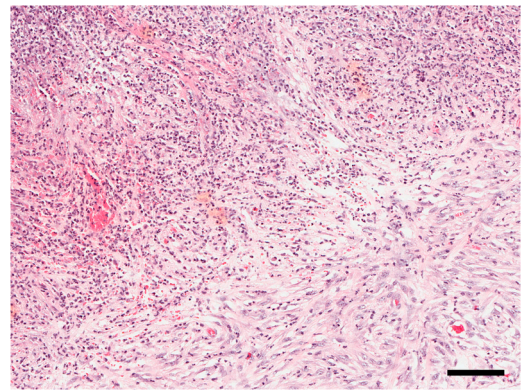rated fewer treatment failures for horses undergoing treatment for equine sarcoids when they are receiving concurrent immuno-modulatory treatment (Haspeslagh et al., 2016). Further studies, exploring the interrelationships with other therapeutic agents and encompassing in situ identification of inflammatory cell subsets are needed to clarify the role and mechanisms involved in this potentially positive effect of inflammation.

The results of this study demonstrated few significant individual associations between clinical and histopathological features and recurrence of equine sarcoids. The presence of multiple sarcoids on an individual horse has previously been associated with recurrence of sarcoids following treatment (Lane, 1977; Vanselow et al., 1988; Carstanjen et al., 1997), although more recent studies have disputed this (Compston et al., 2016). It is possible that horses with larger numbers of sarcoids were underrepresented in this study, as surgical intervention may have been deemed too risky given the degree of tissue resection required. Topical chemotherapy or brachytherapy may have been elected instead. The recurrence prevalence of sarcoids at the surgical site was higher in this study (40.6%) than that found in other studies (14.0%–29.0%), which used similar treatment methods (Martens et al., 2001; Compston et al., 2016; Haspeslagh et al., 2016). Due to the retrospective design of this study, detailed description of the exact surgical techniques from clinical records was not always available, and inclusion of horses undergoing some combination of diode laser/sharp resection was necessary to achieve a reasonable sample size. Many studies with comparatively higher non-recurrence prevalence for electrosurgical techniques (Martens et al., 2001; Compston et al., 2016; Haspeslagh et al., 2016) describe meticulous surgical approaches with non-touch electrosurgical equipment and occasional wound closure, which may have decreased recurrence prevalence. However, the median time to recurrence of sarcoids at the surgical site in one study (7.6 months; Compston et al., 2016) was similar to the 6.8 months determined in the current study, and according to Haspeslagh et al. (2016) sarcoid regression without recurrence for ≥ 6 months following any therapy

may be considered successful. Future studies investigating sarcoid recurrence using larger sample sizes, a prospective design and more detailed evaluation of medical and surgical treatment techniques used, would be beneficial. For example, pre-treatment of sarcoids with pro-inflammatory and immune-stimulatory agents prior to surgical resection, as one treatment group in a randomised controlled trial, may clarify the combined effect these therapeutic modalities may have on recurrence prevalence, given the protective effect of inflammation identified in this study.

The results of this study also showed that the proportion of mares with sarcoid growth at a new site was significantly higher than in stallions/geldings. The reason for this finding is unknown. An association between sex and squamous cell carcinomas (SCC) has been demonstrated with the proportion of SCCs more prevalent in penile and preputial regions of males (Knowles et al., 2016). A higher incidence of lymphoma was found in stallions although this result was considered spurious due to the low number of stallions included in the survey (Knowles et al., 2016). Given that the number of mares ($n = 24$) included in the current study was lower than geldings and stallions ($n = 40$), the possibility of a spurious result may have increased.

The exclusion criteria in this study reduced the number of eligible cases by almost 50%. Twenty-five horses were lost to follow up, and 27 horses had histological sarcoid samples obtained that could not be matched to a specified surgical site, or number of sarcoids surgically removed in the clinical records. A lower number of horses with a complete dataset is currently preferred in equine retrospective pathological studies (Clarke et al., 2014; Ressel et al., 2015). However, following up on horses to determine recurrence of sarcoids over time in this study was prone to reduced statistical power and precision consequent to a low sample size. This may have decreased the ability of this study to determine statistically significant associations and differences and produced wide confidence intervals. Additionally, follow-up by telephone questionnaire rather than using veterinary examination may have resulted in selection and measurement bias, such as loss to follow-up and failure of owners to recall recurred lesions that are occult or small, respectively. However, the use of telephone questionnaires in this study prevented item omission and missing data, which may have been more prevalent if questionnaires had been posted or completed online.

Another source of error in this study was the use of morphological semiquantitative assessment of histopathological samples based on HE staining, which was less quantitative when compared with other approaches, such as immunohistochemical staining for inflammatory cells subclasses. However, currently the morphological identification of different cell types is the most rapid approach, allowing cell categorisation to be more feasible and practical.

Conclusions

Inflammation, specifically located in the superficial neoplasm, was significantly associated with reduced odds of recurrence of equine sarcoids at their original surgical removal site. This may suggest a possible protective role of the inflammatory process in sarcoid recurrence and gives emphasis to the importance of histological analysis of excised sarcoids for prognostic purposes.

Conflict of interest statement

None of the authors have any financial or personal relationships that could inappropriately influence or bias the results of this paper.

Acknowledgements

Appendix A. Supplementary material

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