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Equine sarcoid: BCG immunotherapy compared to cryosurgery in a prospective randomised clinical trial*

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Summary. A total of 30 horses with single or multiple sarcoid tumors of the skin were randomly divided into three treatment groups: (i) cryosurgical treatment, (ii) intralesional immunotherapy with a live BCG vaccine, (iii) intralesional immunotherapy with a BCG cell wall preparation. Complete tumour regression was obtained in all 10 crysurgically treated horses, in 6 of 10 live BCG treated horses, and in 7 of 10 BCG cell wall treated horses. One live BCG and 2 BCG cell wall treated horses showed partial tumour regression of more than 50% of the tumour area. Eleven horses with sarcoid tumours were not eligible for random allocation in the trial because unfavourable site or size of the tumour precluded cryosurgical treatment. These animals were treated with BCG cell wall vaccine except for 1 animal, which was treated with live BCG. In 4 cases this treatment was combined with cytoreductive surgery of the tumour. In this prognostically unfavourable group 8 animals showed complete tumour regression and 3 animals did not respond.

Regression after BCG immunotherapy appeared to correlate with size (larger tumours worse response) and localization of the sarcoid (less favourable results in the limb), and increase in peripheral blood leucocytes after the first injection. Horses with a positive delayed type hypersensitivity reaction to PPD before the start of treatment showed a tendency to more favourable prognosis than PPD negative horses. No correlation was present between regression and single or multiple presence of sarcoids, increase in body temperature after injection of BCG and the formation of specific antibodies to BCG. None of the cured animals have shown tumour recurrence 3 to 40 months following treatment.

Introduction

Equine sarcoid, a fibroblastic skin tumour with a variable epithelial component, is the most frequently found neoplasm in the horse [32, 42, 43]. The tumour does not show metastatic potency, but is notoriously refractory to local treatment. The question raised by Cotchin [7, 8] as to whether sarcoids are real neoplasms is still difficult to answer. Sarcoids demonstrate some characteristics, such as local aggressive growth and proneness to recurrence, which are shared by locally malignant tumours and by tumour-like lesions such as fibromatosis and keloids. Conventional surgical excision is followed by local recurrence in 50% of cases [36]. Due to the high local recurrence rate after surgical excision other therapeutic modalities have been examined. Superficial X-ray irradiation (orthovolt) did not improve prognosis [17]. Radiotherapy (brachytherapy) with ^{198}Au, ^{222}Rn or ^{192}Ir showed good results, but its use in veterinary practice is limited due to the licensing requirements (including special housing facilities) and possible hazards [13, 20, 48, 49]. Hyperthermia, induced by radiofrequency current, was successfully used in three cases of equine sarcoid [19], but only short follow-up periods were available. Cryosurgery has proved to be a safe method of treatment with few complications and a reasonably good success rate of 70% responding animals [14, 21, 26]. However, cryosurgery is a time consuming technique and its use is limited by the localization of some of the tumours.

In the last two decades immunotherapy with Bacillus Calmette-Guérin (BCG) has been widely studied in experimental animal tumour systems [3, 27], in spontaneously occurring tumours in pet [30] and farm animals [18, 23, 24] and in tumours in man [4, 15, 40]. In horses intralesional immunotherapy with BCG was used by Wyman et al. [47] and Murphy et al. [29] for the treatment of sarcoid skin tumours in 2 and 7 horses respectively. Flemming [12] advised BCG treatment for sarcoids smaller than 6 cm. Lesions greater than 6 cm should be reduced in mass by cytoreductive surgery. Recently Schwartzman et al [39] obtained regression in 18 of 20 sarcoids using BCG. Considering the probable viral aetiology of sarcoids [1, 6, 9, 10, 35, 44] it seems likely that equine sarcoids like several viral mouse and rat tumours are immunogenic tumours [28]. In view of the possible reactions of the immune system against tumour cells, this would mean that antigenic sarcoids could form a potential target for immune mechanisms and therefore be suitable for immunotherapy. We compared the best available conventional therapy i.e. repeated freezing and thawing of sarcoids (cryosurgery) with repeated intralesional immunotherapy with live BCG or with BCG cell walls (BCG-CW). BCG-CW was included

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because of the potential risks which have been reported for intralesional administration of live BCG [41].

Materials and methods

Animals. A total of 40 horses (25 mares, 4 stallions and 11 geldings) and 1 donkey admitted to our clinic from November 1980 to January 1984 were included in the study after histological confirmation of the clinical diagnosis "equine sarcoid". Of the 41 animals 24 were Dutch Warmblood Riding horses. The remaining 17 consisted of 4 Friesians, 2 Arabians, 1 Quarterhorse, 1 Fjord, 2 mixed breed, 2 Hackneys, 1 New Forest pony, 1 Trakehner, 2 Welsh ponies and 1 donkey. The age of the animals ranged from 1 to 12 years.

Of the total number, 30 horses were randomly divided over three treatment groups of 10 animals each. One group of animals was treated with repeated intralesional administration of live BCG vaccine (see below), a second group was treated with repeated intralesional administration of BCG-CW material, and the third group of animals was treated with repeated cryosurgery.

Animals were not accepted for the trial if the toal tumour surface exceeded 100 cm^2, or the localization of the tumour prohibited cryosurgical treatment (mostly eyelid tumours). These animals were placed in a separate group ($n=11$) and were treated with BCG-CW ($n=6$) vaccine, live BCG ($n=1$) and BCG-CW combined with surgery ($n=4$). All 41 animals were evaluated after treatment (follow-up for 3 to 40 months).

Anaesthesia and sedation. Before each treatment sedation was performed with acetylpromazine maleate (0.1 mg/kg i.v.) and methadone (0.1 mg/kg i.v.). For cryosurgical treatment induction of anaesthesia was performed with an i.v. administered combination of guajacol glycerine ether (100 mg/kg) and sodium thiopentone (5 mg/kg). After intubation general anaesthesia was obtained with O_2, N_2O and halothane. Most horses treated with immunotherapy were sedated only. The injection of vaccine was performed in a standing position. If necessary, due to tumour localization or to the disposition of the horse, injections were performed in lateral recumbency after administration of guajacol glycerine ether and sodium thiopentone.

Cryosurgery. Cryosurgery was performed at 2–3 weekly intervals with liquid nitrogen delivered through a cryosurgical unit (MVE Cryogenics, Minnesota Valley Engineering, New Prague, Minn., USA) until complete tumour regression was obtained (1–5 cycles). For tumours smaller than 4 cm a probe technique was used, larger tumours were frozen by the spray technique. Each treatment consisted of 2 freeze-thaw cycles. Very large tumours were first frozen, followed by cytoreductive surgery. The residual tumour underwent a double freeze-thaw cycle ($n=5$).

Thermocouple needles were placed in normal tissue immediately beyond the periphery of the sarcoid to ensure that an adequate lethal temperature of at least −25 °C was reached throughout the tumour. When using the spray technique insulating material (styrofoam) was used to protect surrounding tissue.

Immunotherapy. The vaccines used in this study were produced at the Rijks Instiuut voor de Volksgezondheid en Milieuhygiëne (RIVM, Bilthoven, The Netherlands). The BCG vaccine was produced as a concentrated preparation especially intended for use in cancer immunotherapy [22]. The bacteria were grown in homogenous culture with continuous stirring in Ungar medium with 0.05% Tween 80, harvested by centrifugation, resuspended in a stabilizing medium, containing 83 g Haemaccel/l (Haemaccel Polypeptide solution. Hoechst, Amsterdam, The Netherlands), dispensed in 2 ml portions, and lyophilized.

In this study BCG-RIVM lot 077A containing 1.0×10^8 culturable particles in 0.63 mg was used. The content of each vial was resuspended in 2 ml physiological saline solution, shortly before use. The BCG-CW was produced from live BCG-RIVM according to the method described by Ribi [37]. The final preparation contained 3 mg BCG-CW/ml, and was emulsified shortly before use by ultrasound with 3% mineral oil (Drakeol 6VR) and 0.2% Tween 80 in physiological saline.

Each treatment consisted of intralesional injection of 1–14 ml of vaccine. The amount of vaccine depended on the total tumour volume (about 0.25 ml/cm^2, small tumours received propotionally more vaccine than large tumours), taking care to saturate the tumours. Treatment was repeated 14 days, 35 days and 56 days after the first treatment, but was discontinued if tumour regression occurred. More than 4 treatments were given in 5 horses in which tumour growth continued in spite of treatment.

Follow-up. Animals were hospitalised until 3 days after the second treatment, and repeated injections were performed on an outpatient basis. Rectal temperature of the hospitalised animals was recorded daily.

The lesions were photographed at the time of every BCG treatment. Localization and type of tumour, number of tumours and total area of the tumours in cm^2 were recorded. Blood was collected before treatment and at 1, 2 and 3 days after treatment (if the patient was hospitalised) to determine peripheral leucocyte and differential counts. In 21 animals total protein and protein electrophoresis was determined before treatment, in 5 of these 21 the determinations were repeated 14 days after the first treatment. Sera collected before treatment were stored at −20 °C.

Immune reactivity to BCG. Antibodies to BCG were determined by an enzyme linked immunosorbent assay (ELISA). Flat bottomed microtitre plates (Titertek type III, Flow Laboratories) were coated with 100 µl carbonate buffer containing 10 µg/ml purified protein derivate (PPD) of *Mycobacterium tuberculosis* (RIVM, Bilthoven, The Netherlands) and kept overnight at 37 °C. Plates were washed twice for 1 min with tap water containing 0.05% Tween, and then incubated for 10 min with dilution fluid containing 2% rabbit serum and 0.05% Tween in phosphate buffered saline. Rabbit serum was added in order to prevent non-specific binding of horse immunoglobulin to the wall of the microtitre plates. Twofold serial serum dilutions were made in this fluid. Serum dilutions were incubated for 45 min at 37 °C with continuous stirring, then the plates were washed twice. To determine antigen-antibody complexes, 100 µl goat anti-horse serum conjugated with horse radish peroxidase (Cappell, West Chester USA) was used at a dilution of 1 : 1000 and incubated for 45 min at 37 °C with continuous stirring. After washing, the total complex was visualised by adding 100 µl freshly prepared

5-amino salicylic acid and H_2O_2 per well [38]. The reaction product was measured with a multichannel spectrophotometer (Titertek, Multiscan, Amstelstad B.V., Zwanenburg, The Netherlands) at 449 nm after 30 min and expressed as an extinction value. Extinction values greater than 400 at a serum dilution of 1 : 32 were considered to be positive. The median of triplicate values was used. All values were determined on 1 day.

Before treatment delayed type hypersensitivity reaction (DTH) to tuberculin was determined 72 h after i.c. injection of 0.1 ml PPD of *Mycobacterium bovis* (Centraal Diergeneeskundig Instiuut, Lelystad, The Netherlands).

Results

Clinical response to treatment

All 26 tumours treated with cryosurgery showed complete tumour regression (Tables 1 and 2). Complete disappearance of the tumour was generally obtained after 3 to 4

Table 1. Comparison of BCG immunotherapy with cryosurgery: patient characteristics and results

	Animal	Age (years)	Number of sarcoids	Present since (months)	Localization	Total surface (cm^2)	Number of treatments	Follow-up (months)	Result[a]
Cryosurgery	1	8	1	4	leg	3	2[b]	35	+
	2	2	1	3	leg	7	2	28	+
	3	4	7	4	breast abdomen	18	3[b]	23	+
	4	4	1	6	leg	9	3[b]	20	+
	5	8	1	12	leg	84	3[b]	18	+
	6	5	1	23	head	2	5[b]	16	+
	7	2	4	3	leg/head breast	11	3	15	+
	8	11	3	18	abdomen	22	1	14	+
	9	4	1	7	leg	49	5	9	+
	10	15	6	3	anus	8	3	6	+
Live BCG	1	10	2	–	breast shoulder	15	1	29	+
	2	5	1	8	abdomen	16	2	29	+
	3	1	1	6	leg	20	6	27	–
	4	6	1	6	leg	4	3	18	–
	5	4	5	24	legs/head	91	5	18	90%
	6	10	1	84	ear	15	1	16	+
	7	3	4	12	head	30	6	16	+
	8	6	1	3	head	18	5	12	+
	9	12	3	12	leg	56	3	10	–
	10	9	10	60	armpit groin	50	4	6	+
BCG-CW	1	3	5	6	breast abdomen/rump	36	4	40	+
	2	3	1	5	leg	3	2	38	+
	3	2	1	–	leg	4	2	22	+
	4	4	1	6	eye	6	3	20	+
	5	5	1	48	leg	48	3	12	50%
	6	6	1	6	ear	4	3	4	+
	7	8	3	36	head	38	4	11	50%
	8	1	1	8	leg	16	4	5	+
	9	2	1	–	leg	4.5	3	8	+
	10	3	1	5	leg	24	6	5	–

[a] + = complete regression
% = partial regression
– = tumour growth or stationary disease
[b] combination of freezing and incomplete surgery

Table 2. Evaluation of clinical response per tumour

Treatment	*n*[a]	Mean area (cm^2)	Complete regression	Partial regression	No regression
Cryosurgery	26	8.0	26 (100%)		
BCG	29	10.8	24 (83%)		5 (17%)
BCG-CW	16	11.0	11 (69%)	4 (25%)	1 (6%)

[a] number of tumours treated

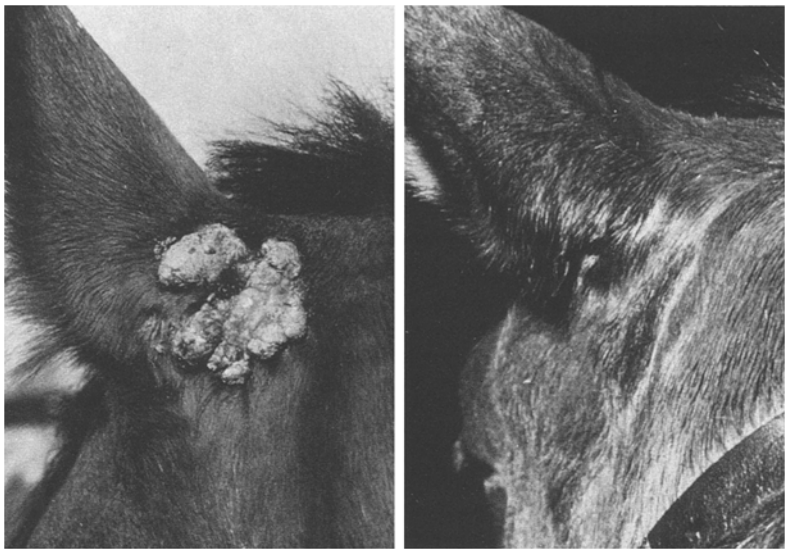

Fig. 1. Equine sarcoid before and 11 months after live BCG treatment

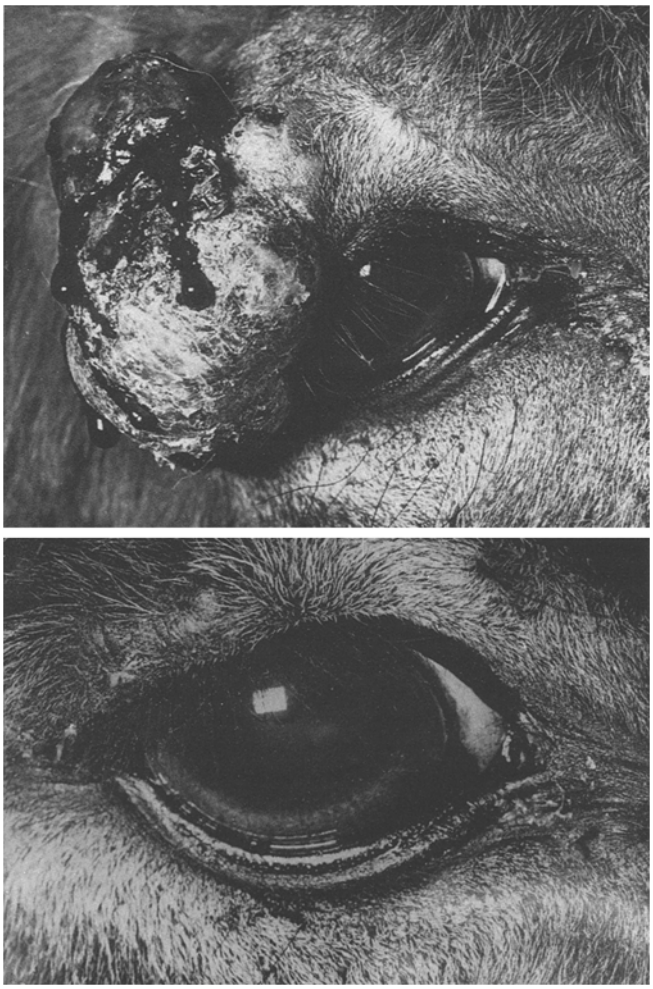

Fig. 2. Equine sarcoid before and 7 months after BCG-CW treatment

freeze-thaw cycles (mean 2.9). In 5 of these 10 animals cytoreductive surgery had been included in the therapy protocol. Regression was completed between 1.5 and 7.5 months (mean 3.3). The range of the follow-up period was 6 to 35 months, (mean 18.4 months).

After intralesional therapy with live BCG, 6 of 10 animals showed complete tumour regression and 1 animal a 90% reduction in tumour size (Table 1, Fig. 1). In 2 animals complete regression was noted after a single intratumoural treatment. Mean time before tumour cure was obtained was 8.6 months (range 2–14 months). Only 3 animals showed no response to treatment. The mean number of injections with live BCG was 3.6. In animals which remained tumour free, the follow-up period ranged from 6 to 29 months (mean 18.0 months). Considering the 29 tumours in this treaetment group, it was found that 83% showed complete regression (Table 2).

After intralesional therapy with BCG-CW, 7 of 10 animals showed complete tumour regression, 2 animals showed a partial response (more than 50% reduction in tumour size) and 1 animal did not respond to therapy (Table 1, Fig. 2). The mean number of injections with BCG-CW vaccine was 3.4. Mean time before tumour cure was obtained was 3.3 months (range 1–7 months). For the animals showing complete regression the follow-up period was 4 to 40 months (mean 19.5). Of the 16 tumours treated in this group 69% regressed completely (Table 2). Partial regression was found in 25% of the tumours. In Table 3 data on the 11 animals treated outside the randomised clinical trial are presented. Of these 11 animals 8 animals showed complete tumour regression, 2 of these after additional surgical treatment; 2 animals showed a partial response. Of these 8 animals 4 were followed for a period of more than 2 years. Mean time for tumour cure was 7 months (2–14 months). There appeared to be no recurrences in animals tumour free after treatment.

Reactions after treatment with BCG vaccine

After intralesional injection all animals developed local swelling followed by ulceration of the injected sarcoids. Local swelling was most pronounced after the second injection, but generally diminished from day 4 to day 7 after injection. In 6 of 31 animals treated with live BCG or BCG-CW vaccine, a more severe reaction was observed, which was not limited to the area of the tumour. In 5 of these 6 animals, with tumours located on the legs, swelling was comparable to that occurring in lymphangitis of the leg, lasting approximately 1 week. For pain relief 2 of

Table 3. Immunotherapy in sarcoid bearing animals not eligible for the trial

Animal	Age	Number of sarcoids	Present since (months)	Localization	Total surface (cm^2)	Treatment	Follow-up (months)	Result[a]
1	8	8	5	head/leg	130	5× cw + surgery	36	+
2	6	7	5	abdomen breast	26	8× cw + surgery	32	+
3	4	1	–	eye	25	4× cw	28	+
4	–	1	4	eye	26	2× live BCG	26	+
5	2	1	–	leg	80	4× cw + surgery	18	±
6	4	12	12	head/leg breast	30	4× cw + surgery	9	±
7	13	1	36	eye	9	4× cw	7	+
8	6	1	6	leg/head	21	2× cw	7	+
9	9	25	48	trunk abdomen	100	6× cw	6	–
10	15	7	–	head/leg trunk/anus	133	2× cw	5	+
11	–	1	–	eye	9	2× cw	3	+

[a] + = complete tumour regression
± = partial regression
– = tumour growth

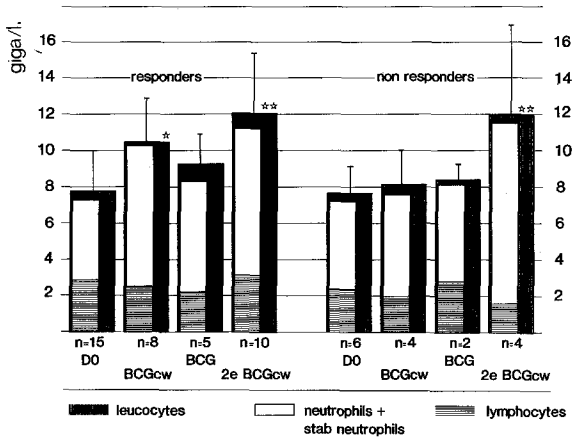

Fig. 3. Changes in peripheral blood leucocytes during the first 3 days after intralesional BCG immunotherapy with BCG-CW or live BCG. The increase in number of leucocytes was most pronounced after the second BCG-CW injection. After the first injection of vaccine a slightly higher leucocytosis was observed in animals in which tumour regression was obtained than in non-responding animals. *, **P < 0.05 res. < 0.01 compared to DO values (Students *t*-test)

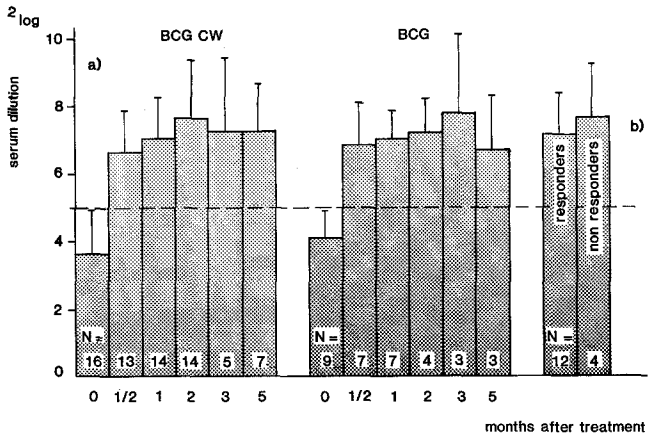

Fig. 4. Antibody production against BCG and BCG immunotherapy in sarcoid bearing horses. **(a)** Mean and SD are presented as ^{2}log of the highest serum dilution which resulted in an extinction of at least 0.400. **(b)** Mean and SD of the maximum ^{2}log dilutions (extinction > 0.400) of sera of 12 animals with complete tumour regression after BCG immunotherapy and sera of 4 nonresponding animals. Animals were intralesionally treated with vaccine on day 0, 14, 35 and 56

these animals were treated with phenylbutazone (4 mg/kg b.i.d.). In 1 of these animals persistent swelling of the leg was still present 10 months after treatment. In the 6th animal with a severe reaction the sarcoid was located on the ear and lymph vessels were swollen 2 days after injection, but the swelling disappeared spontaneously.

Pyrexia on the 1st day and in some cases lasting 2 or 3 days after vaccine injection was a common finding. After the first injection the mean increase in temperature was 0.5 °C (range 0–2.6 °C), after the second injection the mean increase was 1.0 °C (range 0–3.2 °C). Few data of the temperature reaction after the third or fourth injection were available. In these animals temperature rise was comparable to that after the second injection. Comparing the temperature increase of horses treated with BCG-CW or live BCG no difference was present. Anorexia during fever was a common finding.

Leucocyte counts on 21 animals were available. All pre-treatment values were within the normal range. Generally, after intralesional BCG treatment an increase in total peripheral leucocyte count was found, which was even more pronounced after the second injection.

Injection with BCG-CW resulted in a more marked leucocytosis than live BCG (increase 1.9×10^9/l vs 1.2×10^9/l). The increase in leucocytes was caused almost solely by an increase in granulocytes (Fig. 3). Pre-treatment values for total serum protein and protein composition (as determined by routine electrophoresis) were within normal ranges (examined in 21 animals). At several stages after treatment with live BCG or BCG-CW vaccine

Table 4. DTH reaction to PPD before immunotherapy and clinical response to BCG treatment

	n^a	Complete regression	Partial regression	No regression
PPD+	6	5	1	0
PPD−	15	8	1	6

[a] number of animals tested.
Differences are not significant ($p = 0.12$ Fisher's exact test)

Table 5. Total square area of sarcoid in relation to clinical response to BCG immunotherapy

Size (cm^2)	n^a	Complete regression	Partial regression	No regression
< 10	8	7 (87%)	0 (0%)	1 (13%)
10–50	17	12 (70%)	2 (12%)	3 (18%)
> 50	6	2 (33%)	1 (17%)	3 (50%)
	31	21 (68%)	3 (10%)	7 (22%)

[a] number of animals
Tumour regression was more likely to occur in animals with a total tumour area smaller than 50 cm^2 than in animals with a larger total tumour area ($p < 0.05$ Fisher's exact test)

Table 6. Localization of solitary sarcoids in relation to clinical response to immunotherapeutic treatment

Localization	n^a	Complete regression	Partial regression	No regression
Leg	10	5	1	4
Head	8	8	0	0
Abdomen	1	1	0	0

[a] number of animals (= number of tumours)
Solitary sarcoids on the legs appear to respond less favourably to BCG immunotherapy than solitary tumours on head and abdomen ($p < 0.05$ Fisher's exact test)

only minor changes were observed in some horses. All values were within the normal physiological range (data not shown).

Immune reactions to BCG

Specific immune reactivity to BCG was determined by measuring antibody formation to BCG. Based on measurements in sera of 8 normal healthy horses an animal was considered to react positively when the serum could be diluted up to 1:32 or more at an extinction value of 400. Prior to treatment all horses showed titres to BCG below a serum dilution of 1:32. In none of the sera from cryosurgically treated horses could an increase in antibody production to BCG be measured during the first 5 months. All horses treated with BCG developed titres above a dilution of 1:32 (Fig. 4). Even after 2 weeks a mean titre of 1:64 had been reached, and 3 months after the first BCG injection antibody formation reached the highest level (mean 1:128).

DTH reactions to PPD were tested in 21 horses before treatment (Table 4), 6 of which showed a positive reaction.

Correlations of clinical response with various parameters

Results of immunotherapeutic treatment were analysed to determine prognostically important factors. Correlation between clinical response and the presence of single or multiple sarcoids was investigated. No significant difference in response was present between animals with solitary ($n=19$) or multiple tumours ($n=12$). Total surface area was more important for the response to treatment (Table 5). Horses with a total tumour area of less than 50 cm^2 showed a good response in 84% of the cases. Horses with a larger total tumour area responded in only 50% of cases. Localization appeared to be important for response. All of the solitary tumours not located on the legs showed complete regression. Only 60% of the solitary tumours located on the legs responded (Table 6). For multiple tumours the same tendency was present. In the group of 12 animals with multiple tumours, there were 3 non-responders with tumours on the body and the legs (Tables 1 and 3). No correlation was found between the clinical response and the time of existance of the tumour. Horses with sarcoids for less than 6 months and for more than 6 months responded similarly to therapy. Figure 3 shows a tendency for a better prognosis for animals which responded with a higher leucocytosis after the first injection. After the second injection, for both responders and non-responders, a clear leucocytosis was present. Temperature increase (data not shown) was equal in responding an non-responding BCG treated animals. The antibody production to BCG (Fig. 4) was the same in responders and non-responders.

A positive DTH reaction to PPD of *Mycobacterium bovis* before treatment appeared to be favourable. All 6 horses with a positive reaction responded with regression of the tumour after BCG treatment, whereas only 9 of 15 negative horses showed a favourable response (Table 4).

Discussion

In this study immunotherapy with live BCG and BCG-CW has been compared with repeated cryosurgical treatment in a randomised study.

All 10 animals treated with intensive cryosurgery showed complete long lasting tumour cure. These results are better than reported by Joyce [21], Lane [26] and Fretz and Barber [14]. This favourable result was probably obtained by intensive treatment, but it also seems likely that these results were positively influenced by the selection of patients. We selected our patients on both the size of the tumours (total surface smaller than 100 cm^2) and localization (suitable for cryosurgical treatment).

Treatment with BCG-CW resulted in regression in 90% of the cases and total tumour cure in 70% of the horses, which was slightly superior to the results obtained with live BCG (70% and 60% respectively). A fourth group of patients which consisted of animals not eligible for the randomised trial due to tumour size or localization responded in a surprisingly favourable way, as 8 of 11 animals were cured.

Ragland et al. [36] reported a 40% recurrence rate 12 months after surgical removal of the sarcoids. Until now, in none of the BCG treated horses which responded with total tumour cure have recurrences been observed. The mean follow-up time of our patients is now 17 months, so recurrence is at least delayed if not totally prevented, as

suggested by the follow-up period of more than 2 years in 10 patients (Tables 1 and 3). The absence of recurrences might be due to the possible immunity induced by BCG treatment [50] and by cryosurgical treatment. Specific immunological recognition of tumour antigen after cryosurgical treatment has been demonstrated in squamous cell carcinoma of the oral cavity in human patients [11].

Immunogenicity of equine sarcoids has not yet been determined, however indications that equine sarcoids are virus-induced neoplasms are increasing [6]. From laboratory animal tumour systems it is well known that virally induced tumours are in most cases strongly immunogenic [28]. Whether tumour regression by BCG correlates with the immunogenicity of the tumour is still controversial as highly antigenic tumours [31, 34] as well as non-immunogenic tumours [16, 52] have been found to react favourably to BCG immunotherapy.

The toxicity in horses of BCG was shown in a rapid increase in body temperature following BCG injection. However temperature rise was not found in cows after intralesional treatment with live BCG or BCG-CW [23]. Anaphylactic reactions after BCG injection as reported by Winston [46] in horses and by Sparks et al. [41] in man, were not observed in our treatment group.

The results of immunotherapy were analysed against several factors for detection of prognostically important factors. It was found that the square area of sarcoid and the localisation were important for the response to BCG. Animals with a total tumour area of less than 50 cm^2 showed a favourable response in 84% of the cases, whereas only 50% of the animals with a total tumour area larger than 50 cm^2 responded. This less favourable response of animals with a large tumour load is in agreement with data found by Zbar [51] after intralesional BCG treatment of a hepatocellular carcinoma in guinea pigs. Tumours present on the leg responded less favourably to BCG treatment compared to sarcoids located elswhere. The cause for this difference is unclear.

An increase in antibodies to BCG was already observed at day 14 in all horses investigated. Antibodies to BCG were produced in equal amounts by responder and non-responder animals. Thus antibodies to BCG have no prognostic significance. We did not investigate the formation of specific anti-tumour antibodies. A first indication of the presence of specific antibodies to sarcoid antigen was obtained by Watson [45]. Cell mediated immunity against sarcoid cells was demonstrated in vitro by Broström [5]. Future studies are planned to follow the development of the humoral or cellular response against sarcoid antigen during BCG mediated tumour regression.

The immune reaction to a tumour is a complex phenomenon and is the result of series of processes, some favouring tumour rejection, whereas others might promote tumour growth. After the repeated, (in PPD positive horses also after the first) intralesional injections of the sarcoids in our series, a strong DTH reaction was present. Lagrange and Thickstun [25] showed that adding BCG to tumour cells before injection resulted in tumour cell death in BCG immune mice, whereas in non BCG immune mice only a retardation of tumour growth could be obtained. This more favourable response in BCG immune animals is in agreement with the tendency to a more favourable result after BCG injection in PPD positive horses.

Adverse effects like the production of blocking antibodies by B cells or the production of suppressor T cells might also occur [2]. Tumour regression or tumour growth enhancement were reported to be dependent on the dose of BCG [33]. For sarcoids in horses the reactions are in favour of tumour rejection. Tumour enhancement was not seen in the 31 animals studied.

In conclusion, equine, sarcoids are good candidates for cryosurgical treatment and for immunotherapeutic treatment. BCG immunotherapy is more easily performed than cryosurgical treatment and is the therapy of choice in sarcoids not located on the legs. Further studies are needed to identify the immune mechanisms of BCG induced regression of sarcoid skin tumours of horses.

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