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A randomized placebo-controlled double-blinded study comparing oral and subcutaneous administration of mistletoe extract for the treatment of equine sarcoid disease

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Abstract

Background: Equine sarcoids (ES) are the most common cutaneous tumors in equids. Systemic treatment options are sparse. Subcutaneous (SC) injections of *Viscum album* extract (VAE) demonstrate efficacy as a systemic treatment directed against ES.

Objectives/Aim: To critically assess the therapeutic efficacy of orally administered VAE.

Animals: Forty-five ES-affected, privately owned, 3–12 year-old horses.

Methods: A 3-armed randomized placebo-controlled, double-blinded study was conducted in a double-dummy design. Horses were subjected to oral administration and SC injections of either VAE or placebo (VAE oral/placebo SC, VAE SC/placebo oral, placebo oral/placebo SC) over a 7-month treatment period. Primary endpoint was the change of baseline of a composite index of ES number and ES area after 14 months. Second endpoint was the clinical response.

Results: No statistically significant difference in the composite endpoint between the 3 study arms was found. The primary endpoint showed 4 (27%) horses in the VAE oral group with complete ES regression, 3 (21%) in the VAE SC injection group, and 2 (13%) in the placebo group. The clinical response revealed complete or partial regression in 6 horses of the oral VAE group (40%), 4 of the SC injection group (29%), and 4 of the placebo group (25%). Direct comparison of oral VAE and placebo showed an odds ratio, stratified for prognosis of 2.16 (95%-CI: 0.45–10.42) and a *P*-value of 0.336.

Conclusion and Clinical Importance: Oral administration of VAE is well tolerated. No statistically significant difference in the effectiveness of systemic VAE versus placebo against ES was found.

KEYWORDS
equine sarcoid, horse, immunotherapy, mistletoe, randomized trial, *Viscum album* extract

Abbreviations: BCG, Bacillus Calmette-Guérin; BPV, bovine papilloma virus; ES, equine sarcoid; SC, subcutaneous; VAE, *Viscum album* extract.

1 | INTRODUCTION

Equine sarcoid (ES) disease is the most common cutaneous neoplastic condition found in equids and is diagnosed in ~24%–46% of neoplastic equine cutaneous biopsy samples.[1,2] While ES are generally described as being benign, they have the potential for locally aggressive and invasive growth. This can compromise the welfare and use of affected individuals and decrease their economic value. The etiopathogenesis of ES is complex and multifactorial.[3-5]

The treatment of ES is a major therapeutic challenge. This is reflected in the wide variety of treatment options, of which no known treatment method is universally applicable or has led to reliable and lasting success. ES tend to recur, irrespective of the applied treatment.[3] Conventional surgical excision,[6] cryosurgery,[6,7] laser surgery,[8] radiation therapy,[9-11] chemotherapy with active molecules like cisplatin,[12,13] mitomycin C,[14] 5-fluorouracil,[15,16] or bleomycin[17,18] are used for the treatment of ES.

Immunomodulatory treatments can be used either in combination with other modalities or as stand-alone therapies. The intralesional application of Bacillus Calmette-Guérin (BCG) vaccines[6,19] or the topical application of imiquimod[20] trigger local immunomodulatory effects. Cryosurgery[21] and the topical application of bloodroot extracts[22] are likely to induce similar effects. The rationale for systemic ES treatments like autologous vaccines[23,24] or the subcutaneous (SC) administration of mistletoe, that is, *Viscum album* extracts (VAE)[25] is also to induce systemic immunomodulatory effects.

The VAE product used in the present study (Iscador P, Iscador AG, Arlesheim, Switzerland) is currently authorized as a solution for injection in Austria, Germany, Iran, New Zealand, Northern Macedonia, South Korea, Sweden, Switzerland, and the United Arab Emirates to treat human cancer patients with various types of cancer. Adjuvant treatment with SC administered VAE improves the survival of cancer patients by 69%.[26] The antitumoral activity of VAE is attributed to direct and indirect cytotoxic properties,[27-29] induction of apoptosis,[27,30-33] inhibition of angiogenesis,[34] and immunomodulatory mechanisms.[27,35,36] The pharmacologically most studied mistletoe components are viscotoxins and lectins.

Injected VAE is an effective phytotherapeutic agent in the treatment of ES in horses[25,37] and showed promising results in a pilot study with oral administration for the treatment of ES.[38] An observational study on postoperative adjuvant therapy for fibrosarcoma in cats with an oral VAE preparation demonstrated extended survival times and reduced recurrences compared with animals undergoing surgery alone.[39]

The present study aimed to assess the therapeutic efficacy of the oral route of administration for VAE in the treatment of ES. We hypothesized that oral VAE administration is more effective than placebo treatment and as effective as SC injection.

2 | MATERIALS AND METHODS

A 3-armed randomized placebo-controlled double-blinded study was performed in a prospective double-dummy design. The study design was reviewed and approved by the committee for animal experiments of the Canton of Bern, Switzerland, (BE125/2020) and subsequently accepted by all Cantons where study animals were stabled.

All horses were privately owned and stabled in privately owned stables across different regions of Switzerland. A signed informed consent was obtained from all owners. This document clearly stated that horses could receive a placebo and that owners agreed to implement treatment by strictly adhering to the treatment protocol provided. A copy of the consent form is provided in the Data S1.

2.1 | Eligibility criteria and period of recruitment

To be eligible for the study, horses were neither subjected to any specific ES therapy during the 6 months before the trial nor received any other ES therapy than the investigational products during the 14-month study period. Furthermore, horses had to be in an overall good health status and between 3 and 12 years of age upon recruitment. This age range was chosen based on findings from a previous study on VAE treatment against ES[37] and the clinical experience made by some of the co-authors (UB, OC, and CK), which indicate that older horses tend to have a reduced success rate when mistletoe extracts are used as monotherapy. Pregnant mares were excluded, based on the possibility of an altered immune status during pregnancy. To qualify for the study, 3 equine clinicians (AB, OC, CK) experienced with diagnosing ES had to independently agree on the clinical diagnosis of ES, based on a previously validated scoring protocol.[40] Furthermore, a bovine papillomavirus (BPV)-DNA positive swab or a histological confirmation of ES was required for each horse to become enrolled in the study.

The recruitment of study subjects took place between March and December 2021.

2.2 | Sample size

The sample size estimation was based on the outcome of the placebo-treated horses of a previous study on VAE treatment against ES[25] and on the results of a pilot study that included 9 horses and 1 donkey receiving orally administered VAE. This outcome revealed differences in ES numbers (placebo: +0.2 ± 2.5; oral VAE: −2.3 ± 2.1) and affected surface area (removal rate/month: placebo: +0.0145 ± 0.248; oral VAE: −0.234 ± 0.220) over an observation period of 1 year.[38]

A nonparametric Wilcoxon test approach was used to guard against strong deviations from normally distributed data.[41] For a comparison of oral VAE with placebo using equal-sized groups, a power of 80%, and a 2-sided first-type error of 5%, the calculation regarding a composite index of ES number and surface area yielded a sample size of 14 horses per group. Since for the comparison of SC injected VAE versus placebo a similar difference was expected, additional 14 horses were added to the sample size. To account for early dropouts, the number of cases per group was increased to 15 horses, resulting in a total sample size of 45 horses.

2.3 | Baseline data collection

In all horses, the number of ES, their localization, type and size, and the previous treatments were recorded at the beginning of the study. With this basic data, the severity score adapted from Mählmann et al[42] was calculated for each horse (Data S2).

2.4 | Study groups and randomization

All 45 horses were subjected to both oral and SC administrations of either VAE and/or placebo. Horses in the oral or SC VAE group received the study medication by this route and the placebo preparation by the alternative route of administration. Horses in the placebo group received placebo by both routes.

Each horse was assigned to 1 of the following 3 study groups, by stratified block randomization:

1. Oral VAE group: VAE by oral administration and placebo by SC injection.
2. SC VAE group: VAE by SC injection and placebo by oral administration.
3. Placebo (or control) group: placebo by SC injection and by oral administration.

The randomization was blinded. To stratify randomization, each horse was placed in 1 of 3 prognostic categories: good, average, or poor prognosis. To obtain these categories, 2 variables were combined: the age of the horse and the ES severity score. More specifically, for each horse a consensus had to be reached by 3 equine clinicians (AB, OC, and CK) experienced in diagnosing and treating ES disease. The age of the horse was classified as young (2–5 years of age), medium (6–9 years of age), or older (10–12 years of age). The prognosis for expected ES regression was categorized as good, medium, or poor prognosis based on the ES severity score in corresponding severity classes of 3–10 (good), 11–14 (medium), and 15–20 units (poor; Table 1). Horses categorized as having a good prognosis had a maximum of 1 medium age or severity class. The horses categorized as having a medium prognosis had 2 medium or 1 low and 1 high class, and the horses categorized as having a poor prognosis had at least 1 medium and 1 high class.

TABLE 1 The prognosis score combined 2 variables: the age of the horse and the equine sarcoid (ES) severity score adapted from Mählmann et al[42] (see Data S2).

	Severity score		
Age	I (3–10)	II (11–14)	III (15–20)
I (0–5 years)	Good	Good	Medium
II (6–9 years)	Good	Medium	Poor
III (≥10 years)	Medium	Poor	Poor

Note: green = good prognosis score, light blue = medium prognosis score, dark blue = poor prognosis score

Randomization lists of unique noninformative 4-digit codes were created separately for each prognosis stratum by an affiliate of the Society of Clinical Research, Berlin, Germany, who was not involved in any other aspect of the study, using the software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA, 2016). Block-wise randomization of 3 noninformative treatment indices "A," "B," and "C" in permuted blocks of size 3 or 6 was performed, respectively. The lists were given to the responsible person at the Society for Cancer Research, Arlesheim, Switzerland, who decided on the assignment of the treatment indices to 1 of the 3 study groups and who was the only unblinded person involved in the study. Importantly, this unblinded person had no connection with the horses, the owners, or anyone else involved in the practical part of the study.

2.5 | Allocation (concealment mechanism)

Randomization of an eligible horse was requested by the study investigators via e-mail including a screening ID, the horse's name, and the prognosis class to allow stratified assignment. The responsible person at the Society of Cancer Research copied the relevant information in the randomization e-mail together with a randomized code. Finally, a set of study medication boxes labeled with this code and designated for the treatment of this particular horse was dispensed to the investigators.

2.6 | Blinding

Glass ampoules containing VAE or placebo were produced in identical shape, size, and color by Iscador AG, Arlesheim, Switzerland. All ampoules were stored in boxes identically labeled "mistletoe extract series [I or II or 20 mg] or placebo, solution for [oral or SC] administration." On each label, the route of administration was indicated by a large blue (for oral administration) or red (for SC administration) dot. Each horse was treated both orally and by SC injection, with ampoules for both or only 1 kind of administration containing a placebo. This so-called double-dummy design ensured that each horse received the same kind and number of treatments. All study investigators, horse owners, and the statistician analyzing the data were blinded regarding the actual study treatment until the statistical analysis was finalized. The statistical analyses were based on the original uninformative treatment indices A, B, and C and unblinded retrospectively after completing the analyses 4 months after data collection. There were no significant differences in sarcoid type, number, location, or the horses' age between the treatment groups.

2.7 | Exposure

Each horse was either treated with VAE (Iscador AG, Arlesheim, Switzerland), that is, an aqueous extract of *Viscum album ssp. Austriacum*, and/or placebo, that is, a physiological saline solution with 0.9% wt./vol. sodium chloride, for 7 months orally and via SC injection TIW, depending on the study group.

For oral administration, owners were instructed to ensure that the horses took in the liquid (volume of 1 mL) instilled in a piece of bread, apple, or another suitable treat. For the SC injections, all owners were instructed on how to properly administer the solution in the pectoral region. All administrations, except the initial doses, were carried out by the owners.

In horses receiving VAE, irrespective of oral or SC route of administration, this was dosed according to the following scheme for a total of 28 weeks:

1. 2 × series 1, week 1–5: 2× (2× 0.1 mg, 2× 1.0 mg, 3× 10.0 mg).
2. 2 × series 2, week 5–10: 2× (2× 1.0 mg, 2× 10.0 mg, 3× 20.0 mg).
3. 2 × 7 AMpoules 20 mg, week 10–14: 2× (7× 20 mg).
4. 2 × series 1, week 15–19: 2× (2× 0.1 mg, 2× 1.0 mg, 3× 10.0 mg).
5. 2 × series 2, week 19–24: 2× (2× 1.0 mg, 2× 10.0 mg, 3× 20.0 mg).
6. 2 × 7 AMpoules 20 mg, week 24–28: 2× (7× 20 mg).

The dosages refer to the amount of fresh plant material in 1 vial, with each vial containing 1 mL of Iscador.

2.8 | Data collection (follow-up)

During the 7 months of treatment and the 7 months of post-treatment observation, horses were re-examined by the same investigator (AB) at regular monthly (+/−8 days) intervals. Upon each observation, the investigator recorded the number, appearance, and size of all ES. To assess tumor regression or progression during treatment and the observation period, the length, width, and height of each ES was measured, and the total ES area per horse was calculated according to the formula proposed by Thomsen.[43]

At the end of the 14-month study, the treatment response of each horse was assessed by 3 equine clinicians (AB, OC, and CK). For this assessment, digital photographs acquired upon study recruitment and final evaluation at the end of the follow-up period were reviewed. A consensus had to be reached to classify each horse into 1 of the following 5 categories: "complete" or "partial regression," "stable disease," "progression," or "early study termination due to progression." Complete and partial regression were defined as a positive outcome.

2.9 | Statistical analyses

Descriptive statistics for quantitative variables included arithmetic mean, SD, median, first and third quantiles, minimum, and maximum. For categorical data, contingency tables were used, presenting absolute and relative frequencies. Descriptive statistics were generated for all demographic, anamnestic, efficacy, and safety-related variables. Event time data (time to complete regression or intercurrent events (IE)) were visualized using Kaplan-Meier graphs.

The primary efficacy analysis was based on the comparison of the main outcome variable at 14 months after treatment initiation (i.e., immediately after the planned end of observation time). For this purpose, estimates were defined according to the ICH E9 (R1) Addendum. The estimates included the following criteria:

1. "Endpoint" is the composite index according to the PC score of Läuter[44] from the changes in sarcoid total number/total surface area per horse between baseline and the visit 14 months after the start of treatment.
2. "IE" are (a) the need to take additional therapeutic measures or (b) the death of a horse, in both cases because of the deterioration of a horse's health. Three different strategies were used to deal with IEs (a: Treatment Policy Strategy; b: Composite Variable Strategy; c: Hypothetical Strategy), in which the values for the endpoint after an IE, a: as observed; b: replaced by a worst-case value; c: replaced by a value extrapolated from the pre-IE phase; were analyzed to estimate the influence of these events and resulting missing or possibly no longer comparable therapy situations in terms of content.

The horses were not unblinded when an IE occurred. Missing values of ES numbers and/or surface area that occurred at some point during therapy because of missed visits by the investigator were interpolated from previous and subsequent values or estimated from multivariable regression models following the intention-to-treat analysis principle.

The statistical test for differences between the 2 treatment arms was performed using the nonparametric Hodges-Lehmann Aligned Rank Test, which explicitly considers the stratified study design. Together with *P*-values, the standardized effect sizes (i.e., group differences divided by common SD) of the hypothetical strategy are shown. Further sensitivity analyses regarded the trajectories of outcome variables over all study visits using a mixed linear model; or a stepwise variable selection process over all relevant demographic and anamnestic baseline variables to identify prognostically important factors.

Secondary efficacy variables included the individual number, total area, and total volume of all ES, which were analyzed in analogy to the composite score. The horse's overall clinical response after the 14-month study period was analyzed by the Cochran-Mantel-Haenszel χ^2 test for ordered categorical data stratified for prognosis score. Also, the time to complete remission was analyzed by 2-factor Cox proportional hazard regression, including the treatment arm and prognosis score as factors and the number of ES at baseline as covariates.

In a prioritized order of pairwise comparisons of the treatment arms, the placebo and oral VAE treatment groups were compared first. Only if this comparison revealed a statistically significant difference the subsequent comparison between placebo and SC VAE administration could be interpreted in a confirmatory intention. This ensured that the global significance level was maintained without further adjustment of the α-error to multiple testing.

For the assessment of therapy safety, all notable events observed during the VAE administration were documented by the owners. Frequency and hazard rates of events were compared between treatment arms descriptively and by a generalized mixed model, respectively. This included treatment arm and prognosis score as fixed factors and estimating the probability of such events per horse and study visit. All analyses were performed with SAS for Windows version 9.4 (SAS Institute Inc., Cary, NC, USA, 2016).

3 | RESULTS

A total of 83 horses were screened for enrolment, of which 45 horses were found eligible and randomized for treatment. One horse was euthanized 7 days after being enrolled in the study for reasons unrelated to the study and was therefore replaced by another participant (Figure 1).

Of these 45 horses, 14 (31%) received VAE via SC injection; 15 (33%) VAE via oral administration, and 16 (36%) horses received placebo only. Thirteen horses (29%) were Swiss Warmbloods, 6 Franches-Montagnes (13%), 4 Thoroughbreds (9%), 2 Belgian Warmbloods (4%), and 20 horses each belonged to various other breeds (44%). Twenty-five were mares (56%) and 20 were geldings (44%). Twenty-three horses (51%) had already undergone previous unsuccessful treatment or surgery with ES recurrences (Data S3).

All 45 horses finished the treatment period of 7 months without receiving any additional treatment. None of the horses showed or developed any systemic signs indicating poor tolerance of the substance or treatment. Four horses received additional treatment, a combination of surgery, cryo-surgery, and chemotherapy, after the 7-month treatment period. The severe growth of their ES prompted the owners to request additional treatment. Three of these 4 horses belonged to the placebo group, and had a good, medium, and poor initial prognosis, respectively. The other horse received VAE via SC injection and had a poor prognosis. In the oral VAE group, no horse showed signs of worsening during the study period of 14 months.

At baseline, a total of 328 ES were recorded in 45 horses (1–23 ES pro horse, mean 7; oral VAE group, $n = 89$; SC injection VAE group

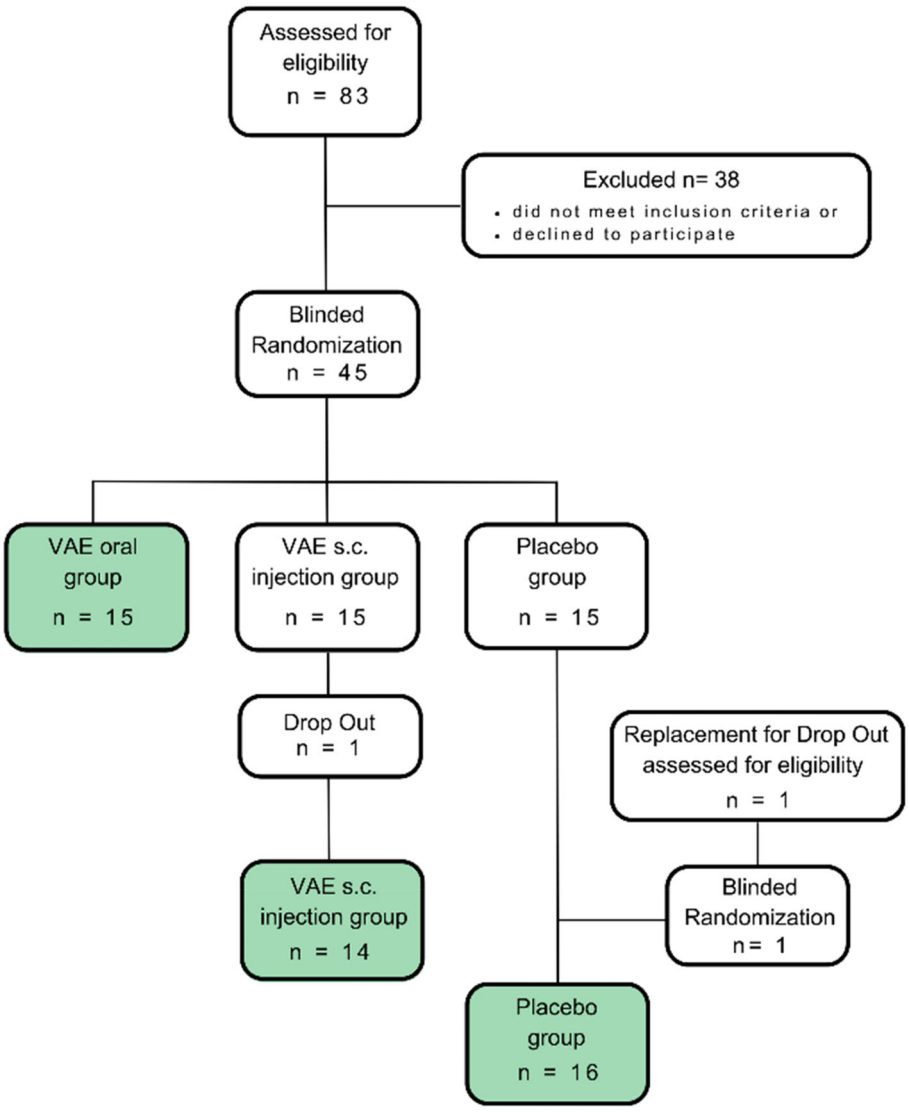

FIGURE 1 Study selection flowchart for the 3-armed randomized placebo-controlled double-blinded study comparing oral and subcutaneous (SC) administration of mistletoe extract (*Viscum album* extract) for the treatment of equine sarcoid disease in client-owned horses.

$n = 117$; control group $n = 122$). The localization of ES tumors was distributed over the following body regions: abdomen (116 ES; 35.4%), inguinal region (47 ES; 14.3%), inner thigh (41 ES; 12.5%), prepuce (38; 11.6%), neck and chest (35 ES; 10.7%), axilla (21 ES; 6.4%), eye (13 ES; 4.1%), head (14 ES; 4.3%), and extremity (3 ES; 0.9%).

The most frequently observed ES type was verrucose, accounting for 147 ES, 44.8% of all ES tumors, followed by occult with 82 ES (25.0%), verrucose-occult 63 ES (19.2%), and nodular 11 ES (3.4%). The remaining mixed ES types, namely "mixed" (i.e., a mixture of more than 2 morphologies), nodular-verrucose, verrucose-fibroblastic, occult-nodular, nodular-fibroblastic, and fibroblastic, each accounted for <2.2%.

In 4 horses (25 ES) of the VAE oral group, in 3 horses (13 ES) of the SC injection VAE group, and in 2 horses (3 ES) in the placebo group all ES lesions had completely disappeared. Partial regression was observed in 2 horses (16 ES) in the VAE oral group, in 1 horse (8 ES) in the VAE SC group, and 3 horses (28 ES) in the placebo group. Progression and partial progression were observed in 5 horses in the VAE oral group, in 7 horses in the VAE SC injection group, and in 8 horses in the placebo group.

The use of VAE, irrespective of the route of administration, did not show a significant reduction in ES number and total surface area per horse in comparison with the placebo group (oral vs. placebo $P = 0.664$; SC injection vs. placebo $P = 0.739$). The blinded clinical assessment of the treatment response after 14 months revealed that complete or partial regression of ES was observed in 6 horses from the oral VAE group (40%), 4 horses of the SC injection group (29%), and 4 horses of the placebo/control group (25%; Figure 2). The direct comparison of oral VAE treatment against placebo for experiencing partial or complete regression of ES did not show a significant effect (odds ratio, stratified for prognosis = 2.16, 95% CI: 0.45–10.42, $P = 0.336$).

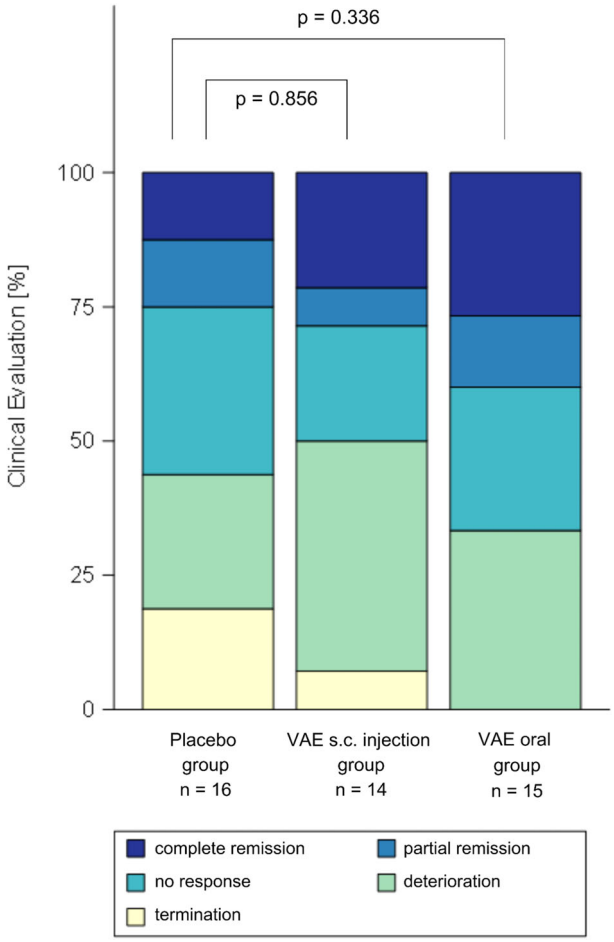

FIGURE 2 Bar graph depicting the outcome of the clinical evaluation after 14 months of treatment. The blinded clinical assessment of the treatment response revealed complete or partial remission of ES in 4 out of 16 horses of the placebo/control group (25%; left bar), and 4 out of 14 horses of the subcutaneous (SC) injection group (29%), compared with 6 of 15 horses of the oral *Viscum album* extract (VAE) group (40%). The direct comparison of oral VAE and placebo showed an odds ratio, stratified for prognosis of 2.16 (95% CI: 0.45–10.42) and a *P*-value of 0.336.

4 | DISCUSSION

Although numerous treatments against ES disease are currently in widespread use, double-blinded placebo-controlled studies critically assessing the efficacy and sustainability are lacking for most of these treatment options.[45] The study design of the present investigation was not only placebo-controlled and randomized but also double-blinded. Therefore, it fulfills every criterion for a rigorously controlled and unbiased investigation. The results of this investigation did not confirm our hypothesis that oral VAE administration is more effective than placebo treatment and as effective as SC injection of VAE.

Spontaneous regression was observed in 2 horses (4 and 8 years of age) of the placebo control group, 1 horse with a good (1 ES, verrucose) and the other with a medium (2 ES, occult-verrucose) prognosis score. These findings are in accordance with previous studies[25,46] where spontaneous tumor regression was documented in young horses with mild disease manifestations. In the study by Berruex, nearly 50% of 3-year-old horses underwent spontaneous regression of occult and verrucose ES over a 5 to 7-year period.[46] In contrast, complete regression was observed in the oral VAE group in 1 horse with a good prognosis (12 ES), 2 horses with a medium prognosis (1 and 4 ES), and 1 horse with a poor prognosis score (8 ES). This highlights that in studies assessing the effectiveness of a therapeutic modality against ES, the potential for spontaneous regression must be considered carefully when calculating appropriate sample sizes for the study cohorts. Especially, when study cohorts are composed of large proportions of younger horses with milder disease manifestations, and in studies with prolonged observation periods.

Oral mucosal drug delivery offers several advantages over injectable routes of drug administration. Because the oral mucosa is highly vascularized, drugs that are absorbed through the oral mucosa directly enter the systemic circulation.[47] This prompts the hypothesis that the prolonged presence of VAE within the oral cavity could activate immune cells, facilitating direct engagement with the systemic

immune system, and ultimately leading to more potent modulation of systemic immune activities. To facilitate the oral administration of VAE, owners were instructed to instill treats like dry bread or apples with VAE or placebo. While some horses might have rapidly swallowed the treats and therefore shortened the contact time with the oral mucosa, treats nonetheless break down rapidly after chewing and release sugars and other components that are readily absorbed within the oral cavity. Furthermore, lectins, which are highly concentrated in VAE, potentially function as mucosal bioadhesins that improve the uptake of particles with immunomodulatory properties.[48]

Although data on the oral bioavailability of mistletoe extracts in horses is lacking, 2 in vitro studies confirm the transport of *Viscum album* lectins across the intestinal mucosa with concurrent stimulation of innate and acquired immunity.[49,50]

In rainbow trout, orally administered VAE was found to have a significant enhancement of antioxidant and innate immune responses.[51] Furthermore, in mice with non-Hodgkin lymphoma, oral administration of mistletoe lectins resulted in a reduced tumor growth rate.[52]

On a practical level, and particularly for medications that require prolonged treatment protocols, horse and owner compliance will increase with oral administration of medications compared with the injectable routes of administration. In addition, certain countries impose restrictions on owners administering SC injections, thereby making oral routes of administration an important alternative. This explains the rationale and incentive for the present investigation.

Another randomized placebo-controlled, blinded study investigated the efficacy of ES treatment with SC applied VAE.[25] In that study, the disease progression was followed in 165 ES lesions across 53 horses. The ages of these 53 horses ranged between 3 and 17 years, of which 6 were more than 12 years old. Making it therefore comparable to our study cohort (3–12 years). The study by Christen-Clottu et al showed ES regression in 41% of horses treated with SC injections of VAE (Iscador P) after 1 year (vs. only 14% of the placebo group) and 63% after 5 years.[25,37] The present study, however, included a total of 328 ES tumors with a mean of 7 ES tumors (range 1–23) per horse. While we measured all ES tumors in each horse, Christen-Clottu et al restricted their analysis to a maximum of 7 ES tumors per horse. Furthermore, in the present study horses were excluded if they had received any ES treatment in the 6 months before the study. The study by Christen et al lacked this exclusion criterium and several horses had recently been subjected to ES treatment just before starting the trial. These differences in study design between the study of Christen-Clottu et al and the present study may partially explain why the results were not replicable.

Most of the current treatment strategies against ES lack a systemic and sustainable effect, which manifests in frequently observed recurrences of ES tumors after therapy.[3,53,54] Conversely, systemic VAE therapy, as suggested by the authors of a previous study,[37] may induce enduring efficacy once successfully implemented. Results from a larger retrospective analysis comparing the efficacy of different treatment modalities and combinations thereof indicate that horses receiving immunomodulating therapies (Imiquimod, BCG, or cryotherapy) in addition to surgery or other conventional treatments had a lower risk of ES tumor recurrence.[21] Likewise, the effectiveness of VAE in the treatment of ES is believed to be induced by an immunomodulatory effect.[36] Therefore, VAE treatment may be used as an adjuvant to other conventional therapies against ES, to increase the effectiveness of topical therapy or control recurrence rates after excisional therapy as suggested by Christen-Clottu et al.[25]

A potential limitation of the present study might be the small sample size. Although predicted to be sufficient by the power analysis, the power analysis was based on the preliminary results of a non-randomized, non-blinded pilot trial with a small cohort and may erroneously have overestimated the efficacy of VAE treatment against ES disease. Therefore, selection bias and favorable assessment may explain an overly positive assessment of the efficacy of oral VAE in the pilot trial, which was not replicable in the more stringent study design of the present investigation. While a simplified approach of using only 2 study groups (oral VAE and placebo) could have increased the number of horses in each group, it would not have provided a comprehensive evaluation of our hypothesis regarding the effectiveness of oral VAE compared to SC VAE. By maintaining separate groups for both oral VAE and SC VAE, we ensured a direct comparison that allowed us to test the specific efficacy of each route of administration. Moreover, the owners, who were not veterinary professionals, carried out all treatments. Although precisely instructed by 1 of the veterinary investigators (AB and OC), this may have led to inconsistencies in the treatment regimen. In an attempt to control adequate administration, ampoules and needles were collected by the end of each month. This ensured a certain degree of supervision, although it did not provide a 100% assurance that horses received all treatments according to protocol. Nonetheless, the overall compliance by owners and horses was very good, with all 45 horses receiving treatment for the full 7 months. Furthermore, we did not consider management and stable changes as a major possible confounding factor in our study design. The environment for 12 horses changed over the 14-month study, which may have influenced their immune status and ES development. Ideally, environmental factors such as this are given more attention in future investigations.

In the present study, the clinical ES diagnosis was based on a validated scoring protocol[40] and backed by a BPV-DNA positive swab or in 1 case a histological confirmation. BPV 1 and 2[55] and a genetic predisposition[56-58] are considered as main factors for the manifestation of ES disease. Recent research indicates that the likelihood of a positive BPV-DNA swab falsely predicting an ES lesion is extremely small, with published positive predictive values of 98% and 100%, sensitivity values of 70% and 88%, and specificity values of 92% and 100%.[59,60] This strategic choice in methodology aimed to balance diagnostic accuracy while mitigating potential influences on tumor behavior.

In this randomized placebo-controlled double-blinded study, the systemic stand-alone treatment with VAE, although well tolerated irrespective of the route of administration, was not significantly more effective against ES disease than treatment with placebo alone.

This study underscores the necessity for rigorously controlled and unbiased investigations to assess the efficacy of various treatments used against ES disease in equine practice. More, and similarly stringent and blinded investigations are needed to advance and optimize ES therapy by providing equine practitioners with evidence-based information regarding the treatment efficacy of available treatment options against ES.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Animal Experimentation Committee of the Canton of Bern, Switzerland (BE125/2020). All animals were privately owned and only participated after an informed consent was signed by the owner.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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