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Research Article

The Effect of Short-Term Treatment with Equine Serum Versus Conditioned Equine or Canine Serum in Three Research Dogs with Spontaneous Keratoconjunctivitis Sicca: A Case Series Pilot Study

Jacqueline Peraza[1], Katrina Jones[1], Kathryn Wotman[1], David D Frisbie[1,2] and Michala de Linde Henriksen[1]*

[1]Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado

[2]Wayne McIlwraith Translational Medicine Institute, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collin, Colorado

***Corresponding Author:** Michala de Linde Henriksen, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado.

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Abstract

Objective: To compare the safety and short-term clinical effect of three serum types [equine serum (ES), 'equine interleukin-1 receptor antagonist protein' serum (eq-IRAP), and canine IRAP serum (ca-IRAP)] on keratoconjunctivitis sicca (KCS) in dogs.

Animals Studied: Three research dogs diagnosed with spontaneous KCS.

Procedures: Each dog was treated for three days, three times a day (TID) in both eyes (OU) with ES, followed by three days of treatment with eq-IRAP TID OU, followed by three days treatment with ca-IRAP TID OU. Clinical parameters (discharge, conjunctival hyperemia, chemosis) were scored on the last treatment day for each serum type. Schirmer tear test (STT) was measured throughout the study. The three different serum types were analyzed with ELISA kits for the concentration of equine interleukin-1 receptor antagonist (eq-IL-1Ra), and ca-IL-1Ra.

Results: All three serum types were well tolerated in the three dogs. Subjectively, dogs treated with ca-IRAP showed improved clinical parameters such as decreased conjunctival hyperemia. Objectively, no differences in STT were seen between the groups ($P > 0.05$). The highest concentration of IRAP was found in ca-IRAP analyzed for ca-IL-1Ra (299.7 pg/mL).

Conclusion: Topical ca-IRAP was noted to have the most significant clinical effect on evaluated parameters. Future long-term studies are needed to confirm the potential anti-inflammatory effect of topical ca-IRAP.

Keywords: Conjunctivitis, Cytokines, Interleukin-1 receptor antagonist protein, Interleukin-1 receptor antagonist, IRAP, KCS.

Introduction

The most common cause of keratoconjunctivitis sicca (KCS) in dogs is immune-mediated damage of the lacrimal glands [1]. West Highland White Terriers, English Bull Dogs, and Pugs are among the most commonly affected breeds [2,3]. A diagnosis of KCS includes low tear production and surface ocular inflammation that clinically can be recognized as mucoid discharge, chemosis, hyperemia of the conjunctiva, and corneal changes such as corneal vascularization, fibrosis, melanosis, and an increased risk for corneal ulceration [2-4].

Sjögren's syndrome (SjS) is a chronic systemic autoimmune disease in humans that is characterized by lymphocytic infiltration of the exocrine glands and epithelium resulting in KCS as well as dry mouth (xerostomia) [5]. The exact immune-pathogenesis is unknown for SjS but it is believed that the proinflammatory cytokine Interleukin-1 (IL-1), especially IL-1beta (IL-1β), is closely involved in the regulation of inflammatory ocular surface molecules. Interleukin-1β activates the migration of leukocytes, which then recruits T-cells and initiates a vicious cycle of a proinflammatory cytokine-mediated disease [6]. A treatment for SjS that has gained recent popularity is the use of a topical IL-1 receptor antagonist (IL-1Ra) [7]. Anakinra (Kineret; Amgen Inc) is an IL-1Ra that suppresses IL-1 mediated inflammation by competitively inhibiting binding of IL-1 alpha (IL-1α) and IL-1β to IL-1 receptor I [7]. Anakinra is approved in the United States for the treatment of rheumatoid ar-

thritis but has also been used off-label to treat other IL-1 mediated inflammatory diseases such as SjS [7]. A randomized clinical trial by Amparo and colleagues revealed that topical 2.5% Anakinra for 12 weeks was safe to use and reduced clinical symptoms of KCS in SjS patients [8].

Interleukin-1 receptor antagonist protein (IRAP) is conditioned serum used in the treatment of equine osteoarthritis to decrease inflammation caused by IL-1 [9,10]. IRAP is produced by incubating whole equine blood with borosilicate glass beads which will cause a release of anti-inflammatory proteins including IL-1Ra, IL-10, IL-4, and Insulin-like growth factor 1 (IGF-1) [9].

To the author's knowledge, no studies have investigated the efficacy of topical IRAP for the treatment of KCS in dogs. The purpose of this pilot study was to evaluate the safety and short-term effect of IRAP in dogs with KCS. We hypothesize that IRAP is safe to use and will have a favorable effect on the clinical signs of KCS compared to the control treatment (hyaluronic acid eye lubrication, OptixCare PLUS) and equine serum (ES). Furthermore, we hypothesize that IRAP made from canine blood (ca-IRAP) will be a more effective anti-inflammatory agent for canine KCS compared to IRAP made from equine blood (eq-IRAP) due to the possibility of species receptor affinity.

Materials and Methods

Dogs

Three research dogs were included in this prospective non-masked pilot study; two dogs were male intact beagles [Dog #1: 11-years of age (15.8kg), Dog #2: 9-years of age (11.7kg)], and the third dog was a male intact hound dog [Dog #3: 11-years of age (15.4kg)]. All three research dogs came from the same research facility and were diagnosed with bilateral KCS four years prior to being included in this study (in 2017) by the research facility veterinarian due to a STT < 15mm/min, mucoid discharge, and conjunctivitis. The three research dogs were initially started on topical cyclosporine 0.2% ophthalmic ointment (Optimmune; Merck Animal Health) OU BID in 2017. Topical cyclosporine had no positive result on tear production or clinical symptoms and the treatment was therefore discontinued. Instead, the three research dogs were treated with topical hyaluronic acid eye lubrication (OptixCare PLUS; Aventix Animal Health Corp.) OU TID as a lifelong treatment. An Institutional Animal Care and Use Committee protocol (IACUC #170.067) was approved for this pilot study.

Ophthalmic examination

An initial complete ophthalmic examination was performed by a board certified-veterinary ophthalmologist (MdLH) on each of the three dogs. The initial ophthalmic examination included Schirmer Tear Test-1 (STT: mm/min), to measure tear production in both eyes (OU) (OptiTech Eye Care; Tarun Enterprises, Prayagraj, Indian). The STT was performed before any eye drops were applied OU. Rebound tonometry (TonoVet Regular; iCare, Helsinki, Finland) of the intraocular pressure (IOP: mmHg) was performed pre-dilation OU. A neuro-ophthalmic examination assessing menace response, pupillary light reflex (direct and indirect), dazzle reflex, and palpebral reflex. The adnexa and anterior segment were evaluated with slit-lamp biomicroscopy (Kowa SL-17; Kowa CO. Ltd). Pharmacological pupil dilation was achieved utilizing 1.0% tropicamide ophthalmic solution (Bausch + Lomb Inc., Laval, Canada) with a single drop (0.05 mL) in each eye. Slit lamp biomicroscopy of the lens was performed when the pupil was dilated. Indirect ophthalmoscopy was performed using a binocular headset (Keeler Instruments Inc. Malvern, PA, USA) and a 28D condensing lens (Volk Optical Inc., Mentor, OH, USA). Photography of the adnexa and anterior segment was performed and documented using a Nikon digital camera (Nikon D750: Nikon Inc., Brighton, MI, USA). Fluorescein stain (Jorgensen Laboratories, Inc., Loveland, CO) was applied OU as the last part of the examination, to assess for corneal defects. The initial ophthalmic examination is henceforth named 'OptixCare' and is used as the control findings.

Sample collection and IRAP preparation

Blood for eq-IRAP was collected from one healthy horse using a single venipuncture. The horse had a physical examination performed by a board-certified equine internist (KW) before blood collection and had no history or signs of systemic disease. Prior to venipuncture, 70% isopropyl alcohol was applied over the jugular furrow to secure sterility. Approximately 65 mL of blood was collected from this horse. Fifty milliliters of blood was placed in a 50 mL conical tube containing glass beads (IRAP tube), provided by Advanced Regenerative Therapies (Fort Collins, Colorado). The IRAP tube is universal and can be used to produce IRAP from all species. The IRAP tubes from this company are not commercially available but can be purchased by contacting the company. The IRAP tube was gently inverted approximately 10 times to mix the blood with the glass beads. The remaining 15 mL of blood was placed in a red top tube (serum) for ES harvesting.

The IRAP tube was incubated at 37°C in an incubator within 30 minutes of blood collection. After 24hours of incubation, the IRAP tube was placed in a centrifuge (Thermo Fisher Scientific, Waltham, MA) and spun at 4000 rpm for 10 minutes with proper counterweights. The tube was then removed from the centrifuge and by using sterile technique, the IRAP was drawn into a 20 mL syringe using an 18-gauge needle under a fume hood. A 0.22 micrometer syringe filter was placed on the 20 mL syringe and utilizing a luer lock coupler, a 6 mL syringe was attached to the output side of the filter. The IRAP was filtered into a 6 mL syringe, and then placed in a 35 mL royal blue top tube (containing no additives). Approximately 25 mL of IRAP was harvested from the equine sample. The eq-IRAP was placed in a -80C freezer for two weeks whereafter it was used in this study.

The remaining 15 mL of blood was placed in red top tubes and centrifuged by the Clinical Pathology Laboratory at Colorado State University and turned into ES by using sterile technique to draw the top layer (serum layer) into a 5 mL syringe using an 18-gauge needle. No micrometer syringe filter or fume hood was used in the process of making ES. The ES was placed in Eppendorf tubes and stored in a -80C freezer for two weeks whereafter it was used in this study.

To acquire ca-IRAP, each of the involved three research dogs had 16.33 mL of blood extracted, to make a total of 50 mL of blood. The blood was combined in a universal IRAP tube from Advanced Regenerative Therapies and inverted 10 times to mix the blood with the glass beads. The IRAP tube was incubated for 24 hours and then centrifuged and harvested in similar fashion as with the eq-IRAP. The ca-IRAP was stored in a -80C freezer for one-week whereafter it was used in this study.

Treatment protocol

The three different serum types that were used in this study were kept in a fridge (4C) while they were being used in this study. The first serum type was ES. All dogs (all eyes) were treated with topical ES 0.1ml OU TID (7am, 1pm7pm) for three days. The second serum type was eq-IRAP: All dogs (all eyes) were treated with topical eq-IRAP 0.1ml OU TID (7am, 1pm, 7pm) for three days. The third serum type was ca-IRAP: All three dogs (all eyes) were treated with topical ca-IRAP 0.1ml OU TID (7am, 1pm, 7pm) for three days. Following the last ophthalmic examination at 7pm in the ca-IRAP treatment protocol, all dogs were restarted on their normal treatment with topical OptixCare PLUS OU TID as a lifelong treatment by the research facility.

There was no washout time between ES to eq-IRAP since both treatments were extracted from equine blood, and the dogs were treated with the blood product (ES) with no known anti-inflammatory properties before starting on the blood product with potential for anti-inflammatory properties (eq-IRAP). A 30-day washout period between the treatment with eq-IRAP to ca-IRAP was decided since this was a switch from an equine to a canine blood product, and both blood products have anti-inflammatory properties. The dogs were treated with topical OptixCare PLUS OU TID during this 30-days washout time.

Clinical parameters

All dogs were examined for four subjective clinical parameters associated with KCS at 7am, 1pm, 7pm, each day; mucoid discharge, blepharospasm, conjunctival chemosis, and conjunctival hyperemia. Lourenco-Martins., *et al.* (2011)'s grading scale for allergic conjunctivitis was used for the subjective grading scores [11]; 0: no signs, 1: mild signs, 2: moderate signs, 3: severe signs. The grading was not masked to the examiners and was performed by a board-certified veterinary ophthalmologist (MdLH) together with a specialty intern in veterinary ophthalmology (JP). All eyes were photographed at every examination for documentation. Three objective clinical parameters were evaluated at 7am and 7pm on the initial examination (OptixCare), and the third day of treatment with ES, eq-IRAP, and ca-IRAP; tonometry measured in mmHg with the TonoVet regular (normal reference value: 15-25 mmHg), [12], STT measured in mm/min (normal reference value > 15mm/min), [13] and fluorescein stain (evaluated as positive or negative for fluorescein stain).

ELISA analysis of ES and IRAP for IL-1Ra concentration

Equine serum (ES), eq-IRAP and ca-IRAP samples that had been in the fridge (4C) for three days were used for this analysis. ES, eq-IRAP and ca-IRAP were analyzed with two different custom ELISA kits, one for equine IL-1Ra and one for canine IL-1Ra (both ELISA kits were purchased from MyBioSource (San Diego, CA, USA). The ELISA reagents were prepared as instructed by the manufacturer. According to MyBioSource, these kits could be used on serum and the three different serum samples were diluted 1:3 [serum to phosphate buffered saline (PBS) ratio] for the analysis. Approximately

25 µL of the diluted sample (ES, eq-IRAP, and ca-IRAP) were placed in each well. One row was set aside on the well for the standard (control) samples. The wells were incubated for 1 hour at 37C. After incubation, 25 µL of detection reagent A (supplied by the ELISA kit) was added to each well. The wells were then incubated for one hour at 37C. Once the incubation period ceased, the samples and standards were aspirated and washed three times with 100 µL of supplied buffer for 1 minute each time (total time 3 minutes). Approximately 25 µL of detection reagent B (supplied by the ELISA manufacturer) was added into each well and incubated for 3 minutes at 37 degrees Celsius. The wells were then aspirated and washed 5 times with a buffer supplied by the manufacturer. About 25 µL of Substrate Solution was added into each well and incubated at 37C for 10 minutes. Once the incubation period ended, 25 µL of Stop solution was added to each well. The plates were placed in an Epoch microplate spectrophotometer (BioTek, Santa Clara, CA) and measurement was conducted immediately at 450 nm. Two standard curves were made, one with equine IL-1Ra and one with canine IL-1Ra concentration on the y-axis and absorbance on the x-axis and a trendline and equation were generated. The absorbance measured for each well was used to obtain concentration (pg/mL) for equine IL-1Ra and canine IL-1Ra in ES, eq-IRAP and ca-IRAP.

Data analysis

Clinical parameters (subjective data) and IL-1Ra concentration (pg/mL) in ES, eq-IRAP and ca-IRAP were analyzed as descriptive data. Objective data (STT and IOP) were analyzed with a one-way analysis of variance analysis (ANOVA). P-values below 0.05 were considered significant. Prism (GraphPad Software; San Diego, CA, USA) was used for statistical analysis.

Results

Clinical parameters

The findings from the initial examination (OptixCare) can be found in figures 1-3 and table 1-3. The initial ophthalmic examination revealed that all three dogs only had KCS (decreased STT, mucoid discharge, hyperemia, and chemosis) in their OS. Dog #3 had the most severe KCS findings OS including severe mucoid discharge and conjunctival hyperemia and mild chemosis as well as corneal pigmentation and vascularization (Figure 3, labeled 'Topical OptixCare'). The result of the subjective clinical parameters from the initial examination (OptixCare), and the third day in each treatment plan for ES, eq-IRAP, and ca-IRAP are shown in Table 1. Figures 1-3 show the eyes of the three dogs from the initial exam (OptixCare), and the third day in each treatment plan for ES, eq-IRAP, and ca-IRAP. By evaluating the data from Table 1, the decrease in conjunctival hyperemia is evident in each dog after three days of treatment with ca-IRAP when compared to the three other treatment options (OptixCare, ES, eq-IRAP). Dog #3 OS was most severely affected by KCS in this study. This eye appeared more comfortable when treated with ca-IRAP, indicated by decreased blepharospasm, after three days of treatment with ca-IRAP compared to the three other treatment options. All three dogs tolerated the three different serum types and none of the dogs developed signs of irritation such as increased blepharospasm or corneal ulceration throughout the study. Although, the eq-IRAP seemed to cause increased mucoid discharge when compared to the two other serum types, especially in Dog #1 (Figure 1, labeled 'Topical equine IRAP').

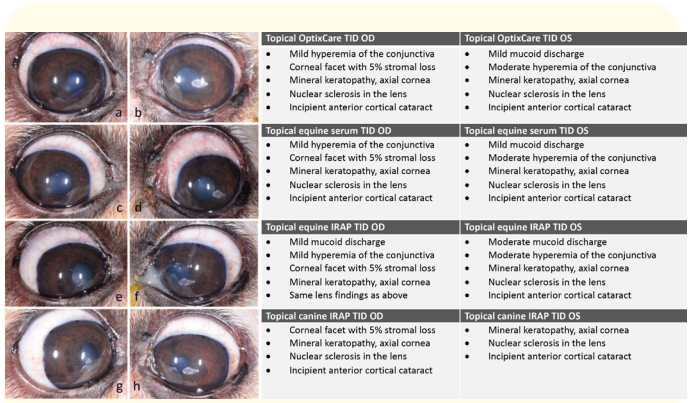

Figure 1: Clinical findings for Dog #1 at the initial exam ('OptixCare'), and the third day in each treatment plan for 'equine serum', equine interleukin-1 receptor antagonist protein ('equine IRAP'), and canine interleukin-1 receptor antagonist protein ('canine IRAP').

STT and IOP

Mean ± standard deviation (SD) from the clinical objective parameters (STT and IOP) from the three dogs' initial examination (OptixCare), and the third day in each treatment plan for ES, eq-IRAP, and ca-IRAP can be seen in table 2 and table 3. No significant differences were found for STT or IOP between the four groups, OU (P = 0.744, P = 0.164, respectively), OD (P = 0.901, P = 0.070, respectively), or OS (P = 0.561, P = 0.195, respectively).

ELISA analysis

The highest concentration of IL-1Ra was found in ca-IRAP analyzed for canine IL-1Ra (299.7 pg/mL), followed by eq-IRAP ana-

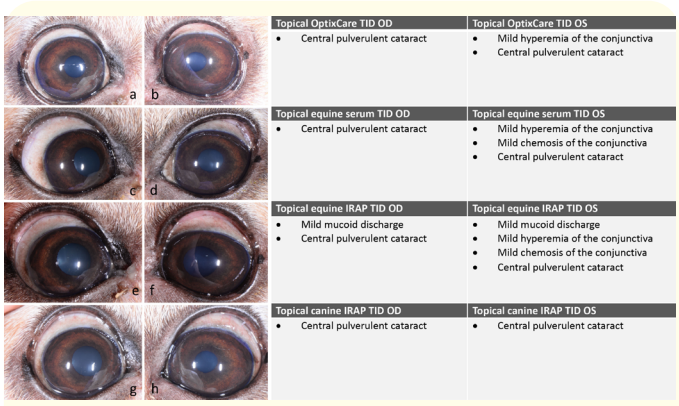

Figure 2: Clinical findings for Dog #2 at the initial exam ('Optix-Care'), and the third day in each treatment plan for 'equine serum', equine interleukin-1 receptor antagonist protein ('equine IRAP'), and canine interleukin-1 receptor antagonist protein ('canine IRAP').

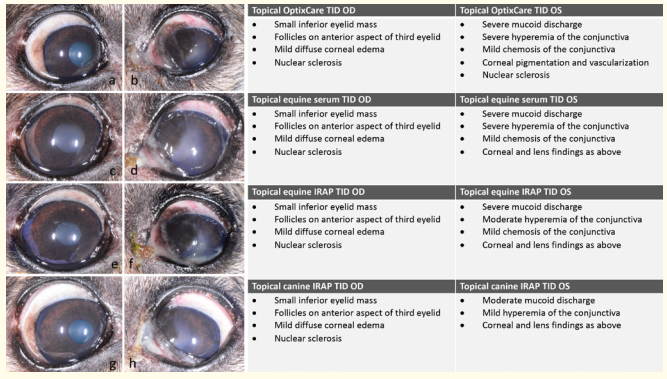

Figure 3: Clinical findings for Dog #3 at the initial exam ('Optix-Care'), and the third day in each treatment plan for 'equine serum', equine interleukin-1 receptor antagonist protein ('equine IRAP'), and canine interleukin-1 receptor antagonist protein ('canine IRAP').

OptixCare		Dog #1				Dog #2				Dog #3			
		ES	Eq-IRAP	Ca-IRAP	OptixCare	ES	Eq-IRAP	Ca-IRAP	OptixCare	ES	Eq-IRAP	Ca-IRAP	
Mucoid discharge	OD	0	0	1	0	0	0	1	0	0	0	0	0
	OS	1	1	2	0	0	0	1	0	3	3	3	2
Blepharospasm	OD	0	0	0	0	0	0	0	0	0	0	0	0
	OS	0	0	0	0	0	0	0	0	2	1	2	0
Chemosis	OD	0	0	0	0	0	0	0	0	0	0	0	0
	OS	0	0	1	0	0	1	1	0	1	1	1	0
Hyperemia	OD	1	1	1	0	0	0	0	0	1	0	0	0
	OS	2	2	2	0	1	1	1	0	3	3	2	1

Table 1: Clinical parameters for the three dogs on their initial exam (OptixCare), and the third day in each treatment plan for equine serum (ES), equine interleukin-1 receptor antagonist protein (eq-IRAP), and canine IRAP (ca-IRAP). The following grading scores were used: 0: no signs, 1: mild signs, 2: moderate signs, 3: severe signs.

STT	Eye(s)	Mean	± SD	[Range]
OptixCare	OU	12.5	4.9	6-19
ES	OU	13.3	5.7	6-20
Eq-IRAP	OU	12.2	5.2	4-17
Ca-IRAP	OU	15.3	5.2	8-20
OptixCare	OD	16.7	2.1	16-19
ES	OD	15.7	1.5	14-17
Eq-IRAP	OD	15	1	14-16
Ca-IRAP	OD	15.3	5	10-20
OptixCare	OS	8.3	2.1	6-10
ES	OS	11	7.8	6-20
Eq-IRAP	OS	9.7	6.7	4-17
Ca-IRAP	OS	15.3	6.5	8-20

Table 2: The mean ± standard deviation (SD) for Schirmer tear test (STT) measured in mm/min collected from OU (both eyes), OD (right eye), and OS (left eye) from their initial exam (OptixCare), and the third day in each treatment plan for equine serum (ES), equine interleukin-1 receptor antagonist protein (eq-IRAP), and canine IRAP (ca-IRAP).

IOP	Eye(s)	Mean	± SD	[Range]
OptixCare	OU	14.5	1.1	13-16
ES	OU	16	2.6	13-20
Eq-IRAP	OU	13.2	2.7	10-18
Ca-IRAP	OU	10.3	2.0	7-13
OptixCare	OD	14.3	1.2	13-15
ES	OD	15.7	2.5	13-18
Eq-IRAP	OD	12	2	10-14
Ca-IRAP	OD	11.3	1.5	10-13
OptixCare	OS	14.7	1.2	14-16
ES	OS	14.7	5	10-20
Eq-IRAP	OS	14.3	3.2	12-18
Ca-IRAP	OS	9.3	2.1	7-11

Table 3: The mean ± standard deviation (SD) for tonometry (intraocular pressure (IOP)) measured in mmHg collected from OU (both eyes), OD (right eye), and OS (left eye) from their initial exam (OptixCare), and the third day in each treatment plan for equine serum (ES), equine interleukin-1 receptor antagonist protein (eq-IRAP), and canine IRAP (ca-IRAP).

lyzed for canine IRAP (183.4 pg/mL), followed by eq-IRAP analyzed for equine IL-1Ra (84.6 pg/mL). No IL-1Ra (0 pg/mL) was found in ES or in ca-IRAP analyzed for equine IL-1Ra (Table 4). OptixCare PLUS was not evaluated.

	Equine IL-1Ra pg/mL	Canine IL-1Ra pg/mL
ES	0	0
eq-IRAP	84.6	183.4
ca-IRAP	0	299.7

Table 4: The three different serum types [equine serum (ES), equine interleukin receptor antagonist protein (eq-IRAP), canine IRAP (ca-IRAP)] were analyzed for their concentration of interleukin-1 receptor antagonist (IL-1Ra). Two different ELISA kits were used, one for equine IL-1Ra and one for canine IL-1Ra. All IL-1Ra concentrations were measured in pg/mL.

Discussion

This pilot study investigated the short-term safety and effect of topical treatment with ES, eq-IRAP and ca-IRAP on KCS in three dogs. To the author's knowledge, no other studies have described the anti-inflammatory effect of these three different serum types for the treatment of canine KCS. This study showed that short-term treatment with topical ES and eq-IRAP did not have a major influence on clinical subjective inflammatory parameters in the three dogs. The clinical subjective inflammatory parameters improved with ca-IRAP with the most significant decrease noted in conjunctival hyperemia.

According to a study by Sawyere and colleagues, analysis of canine autologous conditioned serum, such as IRAP, revealed a higher concentration of IL-1Ra compared to other species [14]. Additionally, the study also found lower concentrations of pro-inflammatory cytokines, such as IL-1β, in canine autologous conditioned serum when compared to other species [14]. These findings could explain why ca-IRAP appeared to be more effective in the treatment of KCS in the three dogs in our study compared to eq-IRAP. Species-specific IL-1 receptors could explain why ca-IRAP showed improved clinical parameters and the lowest IL-1β concentration in tears in this study compared to eq-IRAP and ES. Research into the ligands of IL-1 in various mammalian species has been explored by Koussounadis and colleagues [15]. Koussounadis and colleagues found high levels of variability in the positions of IL-1 receptor binding which may imply that each species has developed its own IL-1 signaling system [15]. More molecular studies are needed to determine the affinity for specific canine and equine IL-1Ra on canine and equine IL-1 receptors. Our study demonstrated that canine IL-1Ra was found in both ca-IRAP (299.7 pg/mL), and eq-IRAP (183.4 pg/mL). Whereas equine IL-1Ra was only found in eq-IRAP (84.6 pg/mL) (Table 4). This could indicate specific species receptor affinity for

equine IL-1Ra. Additionally, the ES in our study did not contain equine nor canine IL-1Ra indicating a lack of anti-inflammatory effect towards IL-1 inflammation.

Currently, there are several commercial eq-IRAP kits available for purchase. However, to our knowledge there is only one company that provides a commercially available IRAP kit that can be used in both equine and canine patients to synthesize conditioned serum (Dechra, Kansas City, MO). The IRAP kits used to produce eq-IRAP and ca-IRAP in our study were provided by a research laboratory and can only be purchased by contacting the laboratory. A possible limitation of using ca-IRAP compared to eq-IRAP in a clinical treatment setting is the amount of blood that can be obtained from one single canine patient. A typical eq-IRAP protocol calls for 50 mL of equine blood to be placed in a tube with glass beads [16]. Obtaining this volume of blood from horses is safe, based on the size of the animal. In comparison, extracting 50 mL of blood from a canine patient may be difficult to harvest depending on canine body weight. Total blood volume of a dog is approximately 85 ml/kg or 8% of total body weight [17]. No more than 1% of the animals' blood volume can be safely collected each day in a two-week period, and a maximum of 10% of the animal's blood volume can be safely collected in a single collection every 14 days [18]. Collecting 50 mL blood for the IRAP process would be safe in a majority of dog breeds that are above 5 kg but would not be possible in dogs that have a body weight below 5 kg including Shih Tzus and Chihuahuas. Furthermore, when treating chronic diseases such as KCS, multiple blood draws long-term would be needed. Little is known about the viability of ca-IRAP when stored for a particular amount of time in a refrigerator (4 degrees Celsius) or freezer (-20 degrees Celsius and -80 degrees Celsius). In a study conducted by Huggins and colleagues, ca-IRAP stored at 4 degrees Celsius, showed a decrease in the stability of the samples by 30 and 90 days [19]. In a study performed by Strauss and colleagues, refrigerated plasma eye drops in both standardized eyedropper bottles and Novelia (multi-dose eyedropper) were safe to use for up to two weeks in dogs with ulcerative keratitis.[20] Given these findings, it is possible that ca-IRAP expiration date can be extended for a longer period if the ca-IRAP sample is kept frozen until use. The ES, eq-IRAP and ca-IRAP used in our study were only stored in the -80C freezer for one week (ca-IRAP) to two weeks (ES and eq-IRAP). We only evaluated the IL-1Ra concentration in ES, eq-IRAP, and ca-IRAP at the end of the study when they had been in the fridge (4C) for three days, and these products could have had a higher level of IL-1Ra immediately following collection and processing. Further research is needed to verify the stability and expiration date of ca-IRAP when frozen.

A limitation of this study was the small population size of dogs and eyes included in the study. Despite the limited number, we were able to show that eq-IRAP and ca-IRAP were safe to use as a topical ophthalmic treatment. Despite a short treatment time, the results of the study demonstrated promising effects of ca-IRAP as an anti-inflammatory treatment for KCS, but long-term studies with larger treatment groups are needed.

Conclusion

In conclusion, topical ca-IRAP appeared to have the most significant clinical effect out of the different serum types studied (ES, eq-IRAP, ca-IRAP). No differences were seen in the dogs STT, however no changes to the STT was expected due to the short-term treatment in this pilot study. Future long-term studies are needed to affirm the anti-inflammatory effect of topical ca-IRAP.

Conflict of Interest

One co-author (DDF) is a shareholder in Advanced Regenerative Therapies, the company that produced the IRAP kits. DDF did not have any input on the interpretation of the results in the study. None of the other authors of this manuscript have any conflict of interest to declare.

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