UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

FIRST EVALUATION OF THE USE OF OCLACITINIB IN THE TREATMENT OF EQUINE ASTHMA PATIENTS NON-RESPONSIVE TO GLUCOCORTICOIDS: EQUINE ASTHMA STAGING

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ORIENTADORA:
Doutora Paula Alexandra Botelho Garcia
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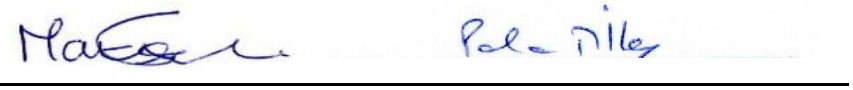
Assinatura: 

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FIRST EVALUATION OF THE USE OF OCLACITINIB IN THE TREATMENT OF EQUINE ASTHMA PATIENT NON-RESPONSIVE TO GLUCOCORTICOIDS: EQUINE ASTHMA STAGING

Resumo

A Síndrome de Asma Equina (SAE) representa uma condição respiratória crónica. Nesta síndrome, os neutrófilos atuam tanto na lesão dos tecidos como nos sinais clínicos. Apesar dos glucocorticoides serem eficazes na maioria das vezes no controlo de episódios críticos de Síndrome de Asma Equina, alguns cavalos apresentam resistência a este tratamento, levando a falhas terapêuticas e efeitos adversos.

O principal objetivo deste estudo piloto foi determinar se a administração oral de Oclacitinib (0,25 mg/kg bwt q.d., comprimidos Apoquel®16mg, Zoetis) melhorava a pontuação Tilley et al. (2012) e a % de neutrófilos no lavado broncoalveolar (LBA) em cavalos diagnosticados por médicos veterinários de 1ª opinião e encaminhados para o hospital escolar de equinos da FMV-ULisboa por SAE resistente a glucocorticoides.

Seis cavalos foram sujeitos a um protocolo de tratamento de 2 semanas com dexametasona. No fim desse protocolo, os sinais clínicos mantiveram-se. Duas semanas depois iniciaram o protocolo do tratamento com oclacictinib em que o receberam diariamente durante 28 dias. Havendo remissão dos sinais clínicos nesse período, a administração passava para dias alternados até ao dia 56. O estadiamento da asma, realizado pelo protocolo de Tilley et al. (2012), e a citologia do LBA foram realizados nos dias 0 e 56. Nos dias 28 e 180, as pontuações clínicas foram registadas.

No dia 56, os 6 cavalos apresentaram uma melhoria média na pontuação total de 69%±34%, com melhorias na pontuação clínica (83%±37%) e endoscópica (100%±0%) em comparação com o dia 0. Dois terços dos cavalos (n=4) não apresentaram diferenças relevantes ao mudar para a administração em dias alternados. Ao dia 56, apenas um cavalo não alcançou o estadio 0 de SAE (pontuação total $\leq$ 4). A citologia do LBA revelou uma predominância de neutrófilos em todas as amostras no dia 0, com uma redução média de 30% após 2 meses de tratamento.

Estes resultados sugerem que o Oclacitinib melhora os sinais clínicos em cavalos com SAE resistente a glucocorticoides quando a exposição a alérgenos ambientais é controlada. Não foram detetadas alterações relevantes nos valores do leucograma ao longo dos 6 meses. Mais estudos são necessários para confirmar a sua eficácia e segurança a longo prazo.

Palavras-chave:

Síndrome de Asma Equina, cavalos, resistência a glucocorticoides, neutrófilos, Oclacitinib.

FIRST EVALUATION OF THE USE OF OCLACITINIB IN THE TREATMENT OF EQUINE ASTHMA PATIENT NON-RESPONSIVE TO GLUCOCORTICOIDS: EQUINE ASTHMA STAGING

Abstract

Equine Asthma Syndrome (EAS) represents a prevalent chronic respiratory condition. In this syndrome, neutrophils have a role in both tissue damage and clinical signs. While most of the times glucocorticoids can control Equine Asthma Syndrome (EAS) exacerbations and are considered the standard treatment, some horses show resistance, leading to therapeutic failure, and side effects.

The main objective of this pilot study was to determine whether orally administered oclacitinib (0.25 mg/kg bwt q.d., Apoquel®16mg tablets, Zoetis1) improved the Tilley et al. (2012) score and the BAL neutrophil % in horses diagnosed with EAS by first-opinion veterinarians and referred to the equine teaching hospital of FMV/ULisboa for glucocorticoid-resistant EAS.

Six privately owned horses initiated a 2 week protocol treatment with dexamethasone (standard treatment). At the end of this protocol, the clinical signs remained. Two weeks after, they began the new treatment protocol with oclacitinib in which they were given the oral dose of oclacitinib (mentioned above) for a period of 28 days. If clinical signs improved by day 28, the dosage continued every other day until day 56. Asthma staging, carried out using the Tilley et al. (2012) protocol, and bronchoalveolar lavage (BAL) cytology were performed on days 0 and 56. On days 28 and 180, Clinical scores were also recorded.

On day 56, six horses showed an average total score improvement of 69%±34%, with significant improvements in clinical (83%±37%) and endoscopic (100%±0%) scores observed at the end of the treatment, when compared to day 0. Two-thirds (n=4) of the horses showed no significant differences when switched to alternate-day dosing. Only one horse did not reach an EAS stage 0 (total score ≤ 4) by day 56. BAL cytology revealed a predominance of neutrophils in all samples on day 0, with a 30% average decrease after two months of treatment.

These findings suggest that oclacitinib improves clinical signs in horses with glucocorticoid-resistant EAS when environmental allergen exposure is controlled. No significant differences in leukogram values were detected over 6 months of follow up period. Further studies are still needed to confirm its efficacy and long-term safety.

Keywords:

Equine Asthma Syndrome, horses, GC resistance, neutrophils, Oclacitinib.

FIRST EVALUATION OF THE USE OF OCLACITINIB IN THE TREATMENT OF EQUINE ASTHMA PATIENT NON-RESPONSIVE TO GLUCOCORTICOIDS: EQUINE ASTHMA STAGING

Resumo alargado

A Síndrome da Asma Equina (SAE) é uma condição respiratória crónica e prevalente que afeta significativamente a saúde e o desempenho dos cavalos. Esta condição é amplamente reconhecida pela sua capacidade de prejudicar a função respiratória dos cavalos, levando a perdas significativas de desempenho físico, especialmente em cavalos de desporto. Uma das principais características patológicas da SAE é a contribuição dos neutrófilos na contribuição tanto para a lesão dos tecidos como para a manifestação de sinais clínicos. Os neutrófilos são um tipo de glóbulos brancos envolvidos na resposta inflamatória do organismo que se acumulam nas vias aéreas e contribuem para a obstrução e hipersensibilidade observadas na SAE. A sua presença é particularmente pronunciada em cavalos que não respondem ao tratamento padrão com glucocorticoides, que são normalmente utilizados para controlar a inflamação associada a esta síndrome. A presença aumentada de neutrófilos nesses casos tem sido intimamente ligada a episódios de agudização da asma, levando a uma diminuição do fluxo de ar, grave desconforto respiratório e, em algumas instâncias, à morte. Por isso, compreender o papel dos neutrófilos na fisiopatologia da SAE é crucial para desenvolver tratamentos mais eficazes para essa condição.

Apesar do impacto significativo da Síndrome da Asma Equina na vida e no desempenho dos cavalos, ainda não existe uma cura definitiva para esta condição. A SAE representa um grande desafio não apenas devido ao seu impacto na saúde equina, mas também devido às limitações das intervenções terapêuticas atuais. O tratamento padrão para a SAE tem sido a administração de glucocorticoides, que são eficazes na maioria das vezes no controlo das crises inflamatórias associadas a esta síndrome. No entanto, um subgrupo de cavalos apresenta resistência à terapia com glucocorticoides, resultando em falha terapêutica e deixando esses animais sem meios eficientes de controlo dos sintomas. Esta resistência aos glucocorticoides não só exacerba os sinais clínicos, mas também aumenta o risco de efeitos adversos decorrentes do uso prolongado dos fármacos, como distúrbios metabólico e imunossupressão. Consequentemente, há uma necessidade urgente de estratégias terapêuticas alternativas que possam efetivamente gerir a SAE, particularmente em casos resistentes aos glucocorticoides, para melhorar a qualidade de vida e o desempenho dos cavalos afetados.

O principal objetivo deste estudo piloto foi avaliar a potencial eficácia de uma opção de

tratamento alternativa, especificamente a administração oral de oclacitinib, na melhoria dos resultados clínicos para cavalos diagnosticados com SAE resistente aos glucocorticoides. O oclacitinib, comumente conhecido pelo nome comercial Apoquel® (Zoetis), é um inibidor da Janus quinase (JAK) que mostrou potencial na modulação de respostas imunológicas e vias inflamatórias. Ao direcionar vias de sinalização específicas envolvidas na inflamação, o oclacitinib pode oferecer uma abordagem inovadora para o maneio da SAE, especialmente em casos onde a terapêutica tradicional com glucocorticoides se mostrou ineficaz. Neste estudo, uma dose de 0,25 mg/kg foi administrada por via oral uma vez ao dia durante um período inicial de 28 dias. Os cavalos presentes no estudo foram selecionados com base no seu diagnóstico de SAE resistente aos glucocorticoides por médicos veterinários de primeira opinião e foram posteriormente encaminhados para o Hospital Escolar de Equinos da FMV/ULisboa para avaliação e tratamento adicionais.

Um total de seis cavalos foram incluídos no estudo. Inicialmente, foram sujeitos a um protocolo de tratamento de 2 semanas com dexametasona (tratamento padrão). No fim desse protocolo, os sinais clínicos mantiveram-se. Duas semanas depois iniciaram o protocolo de tratamento com oclacictinib em que receberam a dose prescrita (0,25 mg/kg) de oclacitinib diariamente durante os primeiros 28 dias. Havendo uma melhoria observada nos sinais clínicos ao final deste período, o regime de administração era ajustado para a mesma dose a cada dois dias durante mais 28 dias, estendendo o tratamento para um total de 56 dias. A avaliação clínica dos cavalos foi conduzida utilizando o protocolo de pontuação de Tilley et al. (2012), que fornece uma avaliação abrangente da gravidade da SAE, incluindo sinais clínicos e inflamação das vias aéreas. Além disso, uma citologia do lavado broncoalveolar foi realizada nos dias 0 e 56 para avaliar a percentagem de neutrófilos presentes nas amostras das vias aéreas, como uma medida da resposta inflamatória. As pontuações clínicas foram registadas adicionalmente nos dias 28 e 180 para monitorizar o progresso dos cavalos ao longo do tempo.

Os resultados deste estudo piloto foram promissores, indicando que o oclacitinib pode oferecer um efeito terapêutico benéfico no maneio da EAS resistente aos glicocorticoides. No dia 56, todos os seis cavalos demonstraram uma melhoria média da pontuação total de 69% ± 34%, destacando uma redução relevante na gravidade dos sinais clínicos associados à EAS. Notaram-se ainda melhorias relevantes nas pontuações clínicas (83% ± 37%) e endoscópicas (100% ± 0%) no final do período do novo tratamento em comparação com os valores iniciais no dia 0 do novo tratamento. Esses achados sugerem que o Oclacitinib pode levar a uma melhoria clínica substancial em cavalos afetados. Além disso, dois terços dos cavalos (n = 4) não apresentaram diferenças relevantes na sua resposta quando a administração foi alterada

para um regime em dias alternados, indicando que a frequência de administração reduzida ainda pode manter os benefícios terapêuticos do medicamento. No entanto, é importante notar que um cavalo não alcançou o estadio SAE 0 (score total $\leq$ 4) até o dia 56, sugerindo variabilidade nas respostas individuais ao tratamento.

A citologia do lavado broncoalveolar (BAL) revelou uma predominância de neutrófilos em todas as amostras no dia 0, consistente com a natureza inflamatória da SAE. Após dois meses de tratamento com oclacitinib, houve uma diminuição média na percentagem de neutrófilos de 30%, indicando uma redução na inflamação das vias aéreas. Esta redução na contagem de neutrófilos vai de encontro com as melhorias clínicas observadas e apoia a hipótese de que o oclacitinib pode modular a resposta inflamatória nas vias aéreas de cavalos com SAE resistente aos glicocorticoides.

Os resultados deste estudo sugerem que o oclacitinib pode ser uma opção de tratamento viável para melhorar os sinais clínicos em cavalos que sofrem de SAE resistente aos glicocorticoides, especialmente quando a exposição a alergenos ambientais é evitada eficazmente. De notar que não foram detetadas mudanças significativas nos valores do leucograma ao longo do período de acompanhamento de 6 meses, indicando que a administração de oclacitinib não originou efeitos hematológicos adversos neste tipo de células. Estes resultados fornecem uma base para investigações futuras sobre a eficácia e o perfil de segurança do oclacitinib como uma opção de maneio a longo prazo para a SAE.

Em conclusão, enquanto que os tratamentos atuais para a Síndrome da Asma Equina envolvem principalmente glucocorticoides, este estudo introduz o oclacitinib como uma potencial alternativa, particularmente para casos que não respondem à terapia convencional. Os resultados promissores deste estudo piloto indicam a necessidade de estudos mais amplos e abrangentes para confirmar a eficácia e o perfil de segurança do oclacitinib no maneio da SAE. Mais estudos serão necessários para determinar o potencial do oclacitinib em tornar-se numa opção terapêutica para esta condição, melhorando, em última análise, a qualidade de vida e os resultados de desempenho para os cavalos afetados por esta síndrome.

Palavras-chave:

Síndrome de Asma Equina, cavalos, resistência a glucocorticoides, neutrófilos, Oclacitinib.

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APP Acute phase protein

AST Antibiotic sensitivity test

BALF Bronchoalveolar lavage fluid

BALFS BALF score

BID Two times a day

CBC Complete blood count

CIART Circadian Associated Repressor Of Transcription

CIC Circulating Immune Complexes

CS Clinical score

CXCL Chemokine ligand

EAS Equine Asthma Syndrome

ECEIM The European College of Equine Internal Medicine

EDTA Ethylenediaminetetraacetic acid

ES Endoscopic score

FMV-ULisboa Faculdade Medicina Veterinária da Universidade de Lisboa

GC Glucocorticoids

GCR Glucocorticoid receptor

GM-CSF Granulocyte-macrophage colony-stimulating factor

IAD Inflammatory Airway Disease

IFN Interferon

Ig Imunoglobulin

IL Interleukin

IV Intravenous

JAK Janus kinase

LPS Lipopolysaccharide

mmEA Mild to Moderate Equine Asthma

MMP Matrix mettallopeptidase

NAD+ Nicotinamide adenine dinucleotide

NET Neutrophil extracellular trap

NK Nuclear factor

NOD2 Nucleotide-binding oligomerization domain 2

PaCO2 Partial pressure of CO2

pH Potential of hydrogen

PO Per os (orally)

RAO Recurrent Airway Obstruction

ROS Reactive Oxygen Species

RT-PCR Real-time polymerase chain reaction

SAA Serum amyloid A

SD Standard deviation

sEA Severe Equine Asthma

SID Once a day

STAT Signal transducer and activator of transcription

TGF Transforming growth factor

Th T helper cells

TIMPS Tissue inhibitors of metalloproteinases

TLR Toll-like receptor

TNF Tumor necrosis factor

TS Total score

USHE Unidade de Segurança e Honras de Estado

XRS X-ray score

Chapter 1. Internship

After concluding my academic studies at the Faculty of Veterinary Medicine, University of Lisbon, I initiated a curricular internship focusing on equine clinical practice and research, as part of my journey towards obtaining a Master's Degree in Veterinary Medicine.

1.1 Internship report

The internship was carried out at the Equine Veterinary Hospital of the GNR Guarda Nacional Republicana (Mounted Police), located at the 4th squad of USHE (Ajuda, Lisbon), having the duration of five months, from September to February. During this period, I accompanied Veterinary Major Hugo Rosa and Veterinary Major Daniela Teixeira, participating in various daily tasks concerning the GNR's horses, which came to a total of 340 animals. These tasks encompassed administering medications, conducting wound care and disinfection, overseeing clinical cases along with their corresponding diagnoses and supplementary tests (such as x-rays, ultrasounds, and endoscopies), providing guidance on orthopedic shoeing, performing basic dentistry procedures, and managing colic cases through procedures like nasogastric intubations, rectal palpations, and fluid therapy, as outlined in tables 1 and 2.

The hospital primarily manages clinical cases related to musculoskeletal issues, with lameness being a prevalent concern. In addition to routine operations, the hospital also engages in managing sports horses and preparing them for equestrian events. Moreover, I was involved in monitoring these horses during their performances, both within the 4th squad's facilities and at external competitions. Specifically, I accompanied the National Cross Country Competition, where several binominals representing GNR competed in show jumping, dressage, and eventing, along with various competitions of the National Jumping Competition.

System/Local	Total
DIGESTIVE	
Medical colic	
Tympanic	12
Pelvic flexure impaction	6
Esophagic obstruction	3
Small Intestine obstrution (lypoma)	1
Surgical colic	1
Dentistry	
Regular dental work	22
Ulcers of the oral cavity	3
MUSCULOSKELETAL	
Frog infections	2
Hoof abscess	18
Cellulitis	3
Traumatic wounds	48
Chronic laminitis	1
Rhabdomyolysis	3
Osteoarthritis	
Proximal interphalangeal joint	4
Distal interphalangeal joint	12
Metacarpophalangeal/Metatarsophalangeal joint	8
Proximal intertarsal joint	2
Distal intertarsal joint	8
Tarsometatarsal joint	12
Digital sheath tenosynovitis	1
Superficial digital flexor tendinitis	2
Deep digital flexor tendinitis	1
Accessory ligament of the deep digital flexor tendon desmitis	2
Suspensory ligament desmitis	2
Calcaneal bursitis	3
Ring bone (4 years old horse)	1
Kissing-spine	5
DERMATOLOGY	
Atopic alergic reactions	6
OFTALMOLOGICAL	
Chronic uveitis	1
Corneal ulcer	5
OTHERS	
Traumatic wounds (not included in the locomotor system)	49

Table 1: Table of pathologies

Procedures	Total
Intra-articular administration	
Proximal interphalangeal joint	2
Distal interphalangeal joint	18
Metacarpophalangeal/Metatarsophalangeal joint	8
Tarsometatarsal joint	14
Sacroiliac joint (ultrasound guided)	12
Back treatments	8
Intramuscular administration	>100
Intravenous administration	>100
Catheter placement	16
Fluid therapy	12
Nasogastric intubation	17
Rectal palpation	15
Perineural nerve block	>60
Mesotherapy	2
Ultrasound	
Distal limb	6
Abdominal	8
Radiography (head, back, limb, thoracic)	>100
Lameness exam	>80
Equestrian performance practices/ Sport horse competitions	53
Orthopaedic shoeing	18
Bandages (Hoof/Distal limb)	45
Wound suture	8
Orthopaedic emergencies	3
Arthroscopic surgery	1
Nasal fracture removal	1
Vaccination/Desparasitation	133
Medical exam of sport horses (Begining of the season)	12
Recovery plans for competion	12
Alimentary plan for sport horses	12
Attendance with oficial veterinary in National Cross Country Competition	1

Table 2: Table of procedures

1.2 Scientific Interaction Project

Through this study, I had the opportunity to participate in a Scientific Interaction Project, organized collaboratively by two high schools of Alenquer and Arruda dos Vinhos, in order to share my work within the field of scientific investigation with younger students, from 16 to 18 years old, making them more familiar with the role of a researcher in the field of veterinary medicine. I presented a five-minute overview of this project, followed by a 30-minute session during which students were invited to ask questions. This interaction provided valuable new insights and constructive feedback, which offered ideas and potential improvements to advance the project.

Appendix 1 ad 2 display the poster for the Scientific Interaction Project, along with the presentations conducted as part of the initiative.

Chapter 2. Introduction

Equine Asthma Syndrome is a common condition in horses for which no definitive treatment has been established. Currently, glucocorticoids are the most commonly used therapy to manage the clinical signs associated with this condition. However, some horses may exhibit resistance to glucocorticoids, resulting in a lack of clinical improvement. Oclacitinib, a JAK1 inhibitor, presents a potential alternative for these cases, as it effectively controls neutrophil airway infiltration, responsible for the clinical manifestations.

2.1 Physiopathology

Equine Asthma Syndrome (EAS) represents a prevalent chronic respiratory condition, significantly impacting the horse's performance. Recent studies have highlighted this equine syndrome as a model for human asthma, given the notable similarities between the two conditions (Bond et al. 2018). The 2016 Revised Consensus Statement delineates EAS into two distinct phenotypes: Severe Equine Asthma (sEA), historically known as Recurrent Airway Obstruction, and Mild to Moderate Equine Asthma (mmEA), previously referred to as Inflammatory Airway Disease (IAD) (Couëtil et al. 2016). It is important to clarify that the progression from Inflammatory Airway Disease (IAD) to Recurrent Airway Obstruction (RAO) should not be considered an inevitable continuum where horses with IAD invariably evolve into RAO over time. Although there is an observed elevated risk of horses exhibiting mild respiratory symptoms progressing to RAO, there is no documented evidence in peer-reviewed literature supporting a direct transition from IAD to RAO based on the current definitions of these conditions (Couëtil et al. 2016). The 2016 Revised Consensus Statement recommends the guidelines ilustrated in figure 1 which detail the clinical presentation and diagnostic confirmation for each of the previous phenotypes.

sEA phenotypes are categorized based on inflammatory triggers into stable-associated sEA, linked to indoor exposure to organic dust from bedding, hay, and straw, and pasture-associated sEA, associated with outdoor exposure to pollen (Holcombe et al. 2001; Klier et al. 2021). This classification, however, does not directly provide treatment strategies or prognostic outcomes, which are largely dependent on the underlying immunological mechanisms.

		Equine Asthma Syndrome	
	Characteristics	IAD (Mild – Moderate Equine Asthma)	RAO or SPRAO (Severe Equine Asthma)
Clinical presentation	Age of onset	Usually young to middle age but can be observed at any age	Usually older than 7 years
	Clinical signs	Occasional coughing, poor performance, no increased respiratory efforts at rest Signs are chronic (at least 4 weeks in duration)	Regular to frequent coughing, exercise intolerance, increased respiratory efforts at rest Signs and severity may vary over time, often limiting activity
	Time course	Often improve spontaneously or with treatment. Risk of recurrence low	Typically last for weeks to months before diagnosis. Usually improves with strict environmental control or treatment. The disease cannot be cured but signs can be controlled
	History	Exposure to stable environment. Genetic susceptibility has not been investigated	Exposure to dust or allergen in stable or at pasture. Some may have a familial history of equine asthma. Clinical signs may be seasonal
Diagnostic confirmation	Airway endoscopy (resting or dynamic)	Excess mucus in tracheobronchial tree (score >1 for racehorses and >2 for sports/pleasure horses). Rule out other differentials	Excess mucus in tracheobronchial tree Rule out other differentials
	Airway cytology	Mild increase in BALF neutrophils, eosinophils, and/or metachromatic cells	Moderate to severe increase in neutrophils
	Lung function	No evidence of airflow limitation based on esophageal balloon catheter technique (DPmax <10 cm H_2O) Airflow limitation detected using sensitive methods Airway hyperresponsiveness	Moderate to severe airflow limitation during disease exacerbation based on esophageal balloon catheter technique (DPmax >15 cm H_2O) Reversible with bronchodilator or environmental change Airway hyperresponsiveness

Figure 1: Clinical presentation and diagnostic confirmation of IAD and RAO. From: Couëtil et al. 2016

EAS is characterized by chronic inflammation driven by a hypersensitive response to environmental allergens, such as dust. This condition likely arises from a combination of genetic predisposition and environmental factors. The interaction between allergens and IgE antibodies on mast cells triggers mast cell degranulation, releasing pro-inflammatory cytokines (Tizard 2014). Clinical manifestations, including coughing, wheezing, and reduced exercise tolerance, stem from bronchoconstriction, airway hyperresponsiveness, mucus overproduction, and airway remodeling (Davis and Sheats 2021).

In human asthma, airway eosinophilia is a fundamental pathogenic feature, with eosinophils releasing mediators that induce airway hyperresponsiveness and mast cell-mediated histamine release. Key mediators include eosinophil peroxidase and major basic protein, which contribute to tissue damage and remodeling, and IL-13, which promotes mucus hypersecretion (Frigas and Gleich 1986; Dalen and Kettle 2001; McBrien and Menzies-Gow. 2017).

Conversely, in equine asthma, significant airway eosinophilia is relatively rare, predominantly observed in young adult horses with moderate asthma (Bond et al. 2018). Hence, a noneosinophilic asthma phenotype, dominated by neutrophil infiltration, is more common in horses. These neutrophils release pro-inflammatory cytokines, exacerbating tissue damage and inflammation (Davis and Sheats 2021). Notably, in severe equine asthma, airway neutrophilia appears resistant to steroid treatment, suggesting an association with Th17 cytokines

rather than the Th2 pathway (Morishima et al. 2011).

2.2 Risk factors

Equine asthma constitutes an allergic reaction triggered by the inhalation of environmental allergens, including but not limited to dust and pollen. Horses housed in stables are at an elevated risk due to the significantly higher concentrations of dust found within such enclosures (Couëtil et al. 2016). The mechanism of action begins with the activation of the innate immune system, specifically through the engagement of Toll-like receptors 2 and 4, along with nucleotide-binding oligomerization domain 2 (NOD2) (Poole and Romberger 2012). Among the myriad components present in dust, lipopolysaccharide (LPS) and *Aspergillus fumigatus* have been identified as predominant risk factors for asthma in both humans and equines, underlining the shared vulnerability to these allergens (Pirie et al. 2001).

2.3 Immune airway mechanisms

2.3.1 Epithelium

The epithelial barrier, comprised of epithelial cells, mucus secreted by epithelial secretory cells, and submucosal glands, serves as the primary defense against foreign particles (Simões et al. 2022). During asthma exacerbation, there is a noted increase in the relative amount of these cells (Bullone et al. 2016). Additionally, immune cells including neutrophils, macrophages, and lymphocytes play a critical role.

Airway epithelial cells provide a mechanical barrier through interactions with fibroblasts and the epithelium and contribute to immunomodulation by secreting chemokines, cytokines, and acute phase proteins (Sha et al. 2004).

Differential responses between asthmatic and healthy horses have been observed, with Tessier et al finding variations in gene expression related to neutrophil chemotaxis and increased transcription of MMP-1, MMP-9, TLR4, and IL-8 in asthmatic horses. Moreover, a decrease in the expression of genes associated with rhythmic processes, specifically CIART, is responsible for a disruption in glucocorticoid response, promoting resistance (Tessier et al. 2017).

2.3.2 The role of neutrophils

Neutrophils play a very important role in the immune system, being one of the major contributors to acute and chronic responses and representing the first line of defense against any

aggression. They contribute through phagocytosis and cytokine, chemokine, protease, reactive oxygen species (ROS), and neutrophil extracellular trap (NET) production (Kolaczkowska and Kubes 2013; Cheng and Palaniyar 2013). Through apoptosis it is possible to regulate their activity, minimizing secondary tissue damage (Fox et al. 2010). Nowadays, it is known that neutrophils have a role in both tissue damage and clinical signs of asthma. However, in the past they weren't considered as a cause effect association instead they were considered just as a secundary reaction. According to Moore et al, an airway neutrophilia is more noteworthy in patients that have no response to inhaled corticosteroids and it has also been linked to asthma exacerbation, decreased airflow and death from asthma (Moore et al. 2014; Ordoñez et al. 2000; Nabe 2020).

Neutrophil influx also happens during acute asthma exacerbation, approximately 4 hours after allergen exposure (Ordoñez et al. 2000). The allergen exposure initiates an increase of cytokynes and chemokynes like IL-17, IL-8, CXCL2 and CXCL10 that will be responsible for the neutrophil influx to the airways (Nocker et al. 1996; Medoff et al. 2002; Singh et al. 2014; Molet et al. 2001). RT-PCR analysis of BALF cells and bronchial epithelium in horses with severe equine asthma (sEA) showed upregulation of IL-1β, IL-8, NF-κB, and TLR4, correlating with neutrophil percentages in BALF (Padoan et al. 2013). IL-8 is one of the most predominant inteuleukins when it comes to neutrophil influx and showed to be increased in BALF in a few hours after changing from grass silage to hay in severely asthmatic horses (Franchini et al. 2000). Neutrophil apoptosis dysregulation, including non-canonical alveolar macrophage phenotypes and IL-17-induced neutrophil viability, sustains neutrophilia.

Noneosinophilic asthma is tied to a Th1/Th17-mediated response, unlike eosinophilic asthma, which involves a Th2-mediated mechanism (Carr et al. 2018). sEA's neutrophilic profile suggests a deviation from the typical Type I hypersensitivity, characterized by eosinophilia. Hypotheses suggested a Type 3 hypersensitivity involvement, yet it does not align with typical features of Type 3 hypersensitivity (Felippe 2016).

Despite the efforts, it is still unclear the precise cytokine profile behind this syndrome. Some reports mention a Th1, a Th2, a Th17 and even a mixed mediated response (Simões et al. 2022). An increase in IL-4 and IL-5 as well as a decrease in IFN-γ expression reinforce a Th2 mediated response (Cordeau et al. 2004). On other hand, a study performed by Ainsworth et al exhibited an increased IFN-γ expression in BALF cells, supporting a Th1 based response.

The cytokine profile associated with this syndrome remains undefined. Hypotheses suggest a combined Th2/Th17 response, attributed to aberrant Th17 cell differentiation (Hulliger et al. 2021). Additionally, reports indicate a general reduction in cytokines, including IL-4, IL-5, IL-13,

and IFN-γ, without a clear dominance of either Th1 or Th2 responses (Kleiber et al. 2005). This variability in cytokine expression suggests that severe equine asthma may encompass various endotypes, akin to human asthma, with a Th17 response implicated in its pathogenesis due to observed neutrophilia in afflicted horses (Simões et al. 2022).

Human asthma's heterogeneity delineates three phenotypes: allergic, non-allergic, and late-onset asthma, each characterized by distinct immune responses. Similar distinctions have been observed in horses (Marti et al. 1991). The first is caractherized by a Th2 mediated response (airway eosinophilia) and according to Feudo et al in severely allergic asthmatic horses there was a Type 1 hypersensitivity in response to intradermal allergen tests, suggesting an association between this allergic condition and other allergic diseases (Feudo et al. 2021). In contrast, the non-allergic phenotype is related to airway neutrophilia, that recognizes a Th1 and Th17 mediated response (Hulliger et al. 2021). Lastly, the late-onset asthma is age associated and is a consequence of a disfunction in the immune system (Hotchkiss et al. 2007).

2.3.3 The importance of IL-17

In both human and equine asthma, IL-17 expression has been closely linked to a neutrophilic response. This cytokine plays a pivotal role in the activation and maintenance of neutrophils within the airways, further contributing to the chronicity of severe equine asthma syndrome. Additionally, the expression of IL-17 is associated with resistance to corticosteroids (Debrue et al. 2005; Murcia et al. 2016).

IL-17, a key pro-inflammatory cytokine, orchestrates the recruitment of neutrophils to sites of inflammation, including the airways. The IL-17 family comprises 6 members (IL-17A - IL17F), The mechanism underlying the neutrophil-recruiting capacity of IL-17A involves the upregulation of neutrophil chemokines, notably CXCL1 and CXCL2 (Wolf et al. 2016; Ramirez-Carrozzi et al. 2011). This effect is compounded by the synergistic action of IL-17A and TNFα, enhancing the production of IL-8 and GM-CSF by airway epithelial cells, as illustrated in figure 2 (Honda et al. 2016). Recent research has established a link between IL-17A presence and corticosteroid resistance (Morishima et al. 2011). In horses afflicted with sEA and exhibiting increased airway neutrophilia, an elevation in IL-17 mRNA expression within BALF cells has been documented (Debrue et al. 2005). Furthermore, IL-17 stimulation has been shown to significantly augment neutrophil viability while simultaneously reducing neutrophil apoptosis (Murcia et al. 2016).

IL-17 is predominantly synthesized by Th17 cells, with their differentiation being facilitated by the combined presence of TGF-β, IL-6, IL-21, and IL-23 (Tizard 2014). T helper (Th) 17 cells

are a new subset of immune cells which play important roles in many diseases like tumor and autoimmune diseases. As a member of CD4+ T cells, Th17 cells express many cytokines such as IL-17A, IL-17E, IL-17F and IL-22. The activation of Th17 cells is one of the pathogenesis paths in human asthma, as these patients express elevated mRNA and IL-17A which seems to be closely related to airway hyperresponsiveness (Yu et al. 2021). Elevated levels of IL-17 protein and mRNA have been detected in sputum, bronchoalveolar lavage fluids, bronchial tissues, peripheral mononuclear cells, and serum of both human and equine asthmatic patients. The concentration of IL-17 correlates with airway hyperresponsiveness and clinical severity, indicating its potential role in the pathogenesis of specific asthma phenotypes (Davis and Sheats 2021).

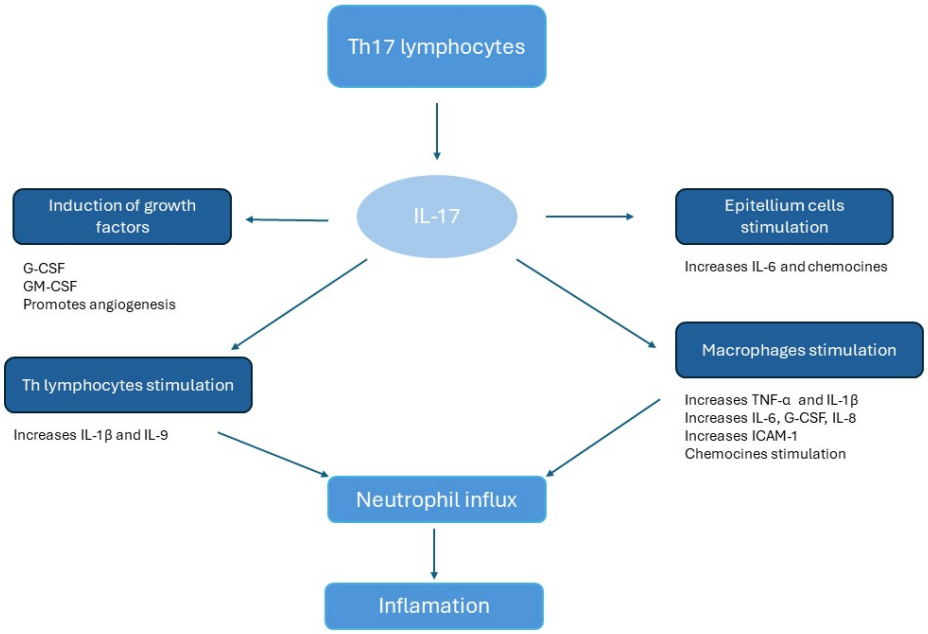

Figure 2: IL-17 properties. Adapted from: Tizard 2014

Corticosteroid therapy's efficacy is notably diminished in the presence of neutrophilic inflammation. Unlike their action on eosinophils, where they promote apoptosis, steroids trigger the mobilization of neutrophils from the bone marrow, decrease the transition of neutrophils from the circulating to the marginating pool, and attenuate neutrophil apoptosis (Saffar et al. 2013; Cox 1995). Wenzel et al have observed that patients exhibiting elevated levels of both neutrophils and eosinophils exhibit more severe clinical symptoms, even when undergoing steroid treatment (Wenzel et al. 1997). Furthermore, it has been reported that steroid therapy does not effectively address airway neutrophilia nor reduce IL-17 and CXCL8 expression in asthmatic patients (Bullens et al. 2006). Murcia and colleagues demonstrated that IL-17-stimulated equine neutrophils exhibit increased IL-8 mRNA expression, which is not reduced by dexam-

ethasone treatment (Murcia et al. 2016). The consequence of airway remodeling, potentially leading to irreversible airflow limitation, has been underscored by studies on sensitized mice. These studies revealed airway remodeling and a direct correlation between its severity and the quantity of $CD4^{+}IL\text{-}17^{+}$ cells, as well as IL-17 levels in the airways (Wang et al. 2010).

While corticosteroids serve as powerful immunosuppressants and anti-inflammatory agents, their mode of action is broadly unspecific. Current therapeutic strategies have yet to yield a targeted treatment for the steroid-resistant phenotype of asthma. Adjusting the Th17 response and managing its signaling pathways may offer a method to mitigate clinical manifestations, suggesting a potential area for future research and therapeutic development (Morishima et al. 2011).

2.4 Diagnosis

2.4.1 Inflammatory biomarkers

For biomarker research in equine asthma, two primary sample collection methods are predominantly employed: Bronchoalveolar Lavage Fluid (BALF) and peripheral blood. Each method presents its own set of advantages. BALF is considered more representative of the pulmonary environment (Bedenice et al. 2008) although it is harder to be used in everyday practice. On the other hand, peripheral blood markers can signal systemic inflammation, though they are less specific (Zareba et al. 2021) and sEA does not typically manifest blood neutrophilia (Couëtil et al. 2016). However, this method offers a non-invasive alternative.

The inflammatory biomarkers used in equine asthma are:

1. Acute Phase Proteins (APP): Haptoglobin has been associated with airway remodeling. Lavoie-Lamoureux and colleagues reported elevated serum haptoglobin levels in horses with sEA, though increases are also observed in controls post antigen exposure, indicating systemic reflection of airway inflammation (Lavoie-Lamoureux et al. 2012). Serum amyloid A (SAA) is associated with neutrophil recruitment in human asthma, and in horses with severe asthma, its levels rise for seven days after the antigen exposure (Lavoie-Lamoureux et al. 2012). These proteins despite their utility to identify systemic inflammation, are not disease specific (Simões et al. 2022).
2. Circulating Immune Complexes (CIC): Increased levels of CIC have been reported in sEA (Nied´zwied´z et al. 2014; Slowikowska et al. 2021). It has been found that they can be potentially useful in monitoring treatment efficacy (Slowikowska et al. 2021). However, further research is required to validate their diagnostic utility.
3. Matrix Metalloproteinases (MMPs), Tissue Inhibitors of Metalloproteinases (TIMPs) and

MMPs/TIMPs ratio: Derived from bronchoalveolar lavage, these biomarkers aid in tracking disease progression and corticosteroid response (Barton et al. 2015). MMPs facilitate collagen degradation (Doren 2015) and TIMPs can promote fibrosis. MMPs also help regulate physiological barriers and modulate cytokine and chemokine activity, establishing gradients for leukocyte migration into inflamed tissue (Naito and Yoshikawa 2005). Glucocorticoid treatment has been shown to reduce MMP-2, MMP-9, TIMP-1, and TIMP-2 levels according to Barton et al.

4. IFN-γ: Levels are elevated in the BALF of horses with sEA, enabling differentiation between asthmatic and non-asthmatic animals (Woodrow et al. 2020)

In summary, while systemic markers are more accessible through blood samples, their specificity for pulmonary disease is relatively low. This creates the need to exclude other unrelated or potentially subclinical conditions. Conversely, BALF markers offer greater diagnostic accuracy for airway disease, given the possibility of performing bronchoalveolar lavage in standing sedated horses, making them more suited for specific investigations of respiratory pathologies.

2.4.2 Clinical signs and history

Horses diagnosed with Equine Asthma Syndrome (EAS) often have a recurrent respiratory disease history, which may be linked to specific environmental factors. These factors tend to be seasonal, most commonly observed in spring and autumn, and some horses may exhibit symptoms during or after physical exertion (Simões et al. 2022).

Clinical manifestations of airway inflammation, hyperreactivity, and obstruction in horses include coughing, which is frequently the initial sign noted by owners, exercise intolerance, increased respiratory effort at rest, and nasal discharge (Rettmer et al. 2020; Feudo et al. 2021). The intensity and persistence of these symptoms are often directly correlated with the severity of the inflammatory process (Bedenice et al. 2008; Janssen et al. 2022).

Mucus accumulation within the tracheobronchial tree is another common sign, detectable through auscultation over the tracheal area (Gerber et al. 2000). Thoracic auscultation may reveal increased bronchovesicular sounds, end-expiratory wheezes from airway narrowing, and inspiratory crackles as collapsed airways reopen (Simões and Tilley 2023). In severe cases, horses may also experience weight loss and cachexia due to the decreased food intake and the increased energy expenditure needed to overcome expiratory obstruction (Mazan et al. 2004).

2.4.3 Imaging

Radiographic evaluations of horses with severe Equine Asthma (sEA) may show lung pattern changes, such as interstitial and bronchial interstitial lung patterns, and tracheal, bronchial wall thickening and increased bronchovascularity (Tilley et al. 2012; Bakos 2008). However, X-rays have limitations and cannot detect mild lung inflammation (Simões et al. 2019).

2.4.4 Endoscopy

Endoscopic examinations typically reveal mucus in the trachea and bronchi, alongside carina thickening (Simões and Tilley 2023). The quantity and quality of tracheal secretions are indicative of clinical severity (Costa et al. 2000). While secretion amounts may decrease during disease remission, mucus scores alone cannot differentiate affected horses from healthy ones (Simões et al. 2019; Rettmer et al. 2020), highlighting the need to integrate various diagnostic methods alongside clinical history for accurate diagnosis.

2.4.5 Lung function tests

Lung function tests are the gold standard diagnostic for asthma in both humans and horses (Simões and Tilley 2023). Arterial blood gas allows an evaluation of gas exchange in the lungs and horses with severe equine asthma usually present hypoxemia, lower values of pH and increased values of PaCO2. The evaluation of pleural pressure, using an oesophageal ballon catheter, allows a good disease diagnosis in an ambulatory setting, being considered the gold standard for sEA (Simões and Tilley 2023). However, the change in pleural pressure alone can not differ healthy animals from severely asthmatics in remission, still if combined with histamine bronchoprovocation, its sensitivity improves and it can be used as a valuable tool for rating respiratory function (Doucet et al. 1991).

These tests can be used in combination with a bronchodilator. In case of severe compromise of baseline pulmonary function it is expected that within 10 min, a 50% improvement of airway resistance should occur (Mazan et al. 2004; Mazan and Hoffman 2003). They can also be used with a histamine challenge in order to check the airway response to a bronchoconstrictor stimulus. In cases of asthma exacerbation, they are expected to induce airway hyperreactivity and reversible narrowing of airways (Mazan and Hoffman 2003).

2.4.6 Cytology

Cytological analysis is an effective method for diagnosing and monitoring sEA, providing insights into the airways' inflammatory status. Although various sample collection methods exist,

BALF cytology remains the most precise for cell counting. The cytological profile of a horse with a sEA is predominantly marked by neutrophilia, with decreased percentages of macrophages and lymphocytes (Simões and Tilley 2023). A higher neutrophil percentage correlates with disease severity, coughing frequency, and worse mucus scores, thus serving as a criterion for sEA severity classification (Tilley et al. 2012). For reference, healthy horses should have BALF cytology values of $\leq$ 5% neutrophils and $\leq$ 1% eosinophils in a 250ml infusion volume (Couëtil et al. 2016).

However, BALF cytology should not be the sole diagnostic criterion for SEA, as horses displaying increased respiratory effort may not exhibit BALF neutrophilia. Moreover, corticosteroid therapy can alter the cellular population in the airways, complicating the diagnostic accuracy of cytology (Bullone and Lavoie 2017).

2.5 Equine Asthma Staging

Proper staging of asthmatic horses is critical for formulating an effective treatment plan and enhancing the likelihood of a positive therapeutic outcome. Staging also facilitates the monitoring of disease progression by assessing the horse's current condition. Clinical history, observable clinical signs, cough frequency, nasal flare, and abdominal lift, alongside diagnostic measures such as thoracic X-rays, endoscopy and BALF cytology are essential for EA staging (Simões and Tilley 2023). However, this approach has limitations, including the need for hospital-based procedures and the absence of lung function evaluation, which is a significant diagnostic indicator reported by the ECEIM 2016 consensus on inflammatory airway disease (Couëtil et al. 2016).

2.6 Current treatment

Equine Asthma Syndrome (EAS) is a chronic inflammatory condition without a definitive cure. Nonetheless, management strategies, including medication and environmental adjustments, can mitigate symptoms and prevent crises. Glucocorticoids (GC) are effective in improving conditions within days but offer limited benefits post-treatment cessation. Thus, combining GC treatment with dietary and environmental modifications to minimize antigen exposure is advisable (Mainguy-Seers and Lavoie 2021) and the concurrent use of β2-adrenergic agonists with GC is recommended for optimal bronchodilator efficacy (Abraham et al. 2002). Despite these measures, achieving complete antigen avoidance can be challenging, leading to recurrent exacerbation (Simões and Tilley 2023).

Systemic GC administration offers greater efficacy than inhalation routes due to mucus accu-

mulation, bronchospasm, and coughing, which impede lower airway deposition (Jocelyn 2018). Dexamethasone, the most researched glucocorticoid for asthma treatment, shows clinical improvement within three days, whether administered intravenously (0.04-0.1 mg/kg q24h) (Picandet et al. 2003) or orally (0.05 mg/kg q24h) (Leclere et al. 2010). Benefits include enhanced clinical scores, lung function, reduced mucus and cough frequency (Robinson et al. 2003), and at a dose of 0.1 mg/kg IV q24h for five days, it prevents downregulation of clenbuterol-induced β2-adrenergic receptors.

Inhaled beclomethasone dipropionate (1320 µg q12h for 7 days) may alleviate airway obstruction but is less effective than systemic dexamethasone (0.1 mg/kg IV q24h) (Rush et al. 1998). Fluticasone, with low oral bioavailability, minimizes systemic side effects (Baptist and Reddy 2009), improving lung function in antigenic environments within a month at a dosage of 2000 µg q12h (Leclere et al. 2012). Higher doses (6 mg BID for 7 days) are preventive against exacerbations (Robinson et al. 2009).

2.6.1 Effects of glucocorticoids on BALF cells

GC's potent anti-inflammatory properties have limited impact on airway neutrophilia. Nonetheless, dexamethasone can reduce airway neutrophilia when antigenic exposure ceases (Barussi et al. 2016). Dexamethasone is the most common GC used in asthma patients since it has a greater effect when compared to other systemic GC like prednisolone. Prednisolone, combined with antigen avoidance, shows a more significant reduction in airway neutrophilia than environmental changes alone (Jackson et al. 2000).

With the results of former studies, it was possible to conclude that it is very important to control the environment in wich horses with sEA are because GC alone fail to reduce airway neutrophilia but together with antigenic avoidance, an improvement of airway neutrophilic inflammation is possible (Mainguy-Seers and Lavoie 2021).

2.6.2 Adverse effects

Laminitis is a known side effect of GC treatment, though it is not commonly reported in asthmatic horses treated systemically with the recommended dosage. However, predisposed horses may still face risks (Mainguy-Seers and Lavoie 2021). Another concern is immunosuppression, which is generally not associated with short-term treatments. Nevertheless following prolonged glucocorticoid treatment, respiratory fungal infections can appear (Marsella et al. 2023). Decreases in lymphocytes and eosinophils, alongside increases in neutrophils, have been observed, attributed to their mobilization from the marginated pool (Picandet et al. 2003;

Carakostas et al. 1981).

2.6.3 Lack of response to the GC treatment

The cellular mechanisms of GC resistance in horses aren't still fully known. In human asthma, the overexpression of pro-inflammatory transcription factors and of the GCR-β (Barnes 2013; Hew and Chung 2010) and several clinical factors are responsible for the modulation of GC sensitivity.

Unsuccessful treatment responses may arise from continued antigen exposure, empirical treatment approaches, noncompliance by owners, incorrect administration techniques, improper dosing, and misdiagnosis (Simões and Tilley 2023; Mainguy-Seers and Lavoie 2021).

Another important factor associated to glucocorticoid insensitivy described in human asthma is aging-related mechanisms such as hormone production, immune cell infiltration, lung function, cellular senescence and NAD+ Metabolism (Ford et al. 2023).

2.7 Oclacitinib

Oclacitinib has been available in the veterinary medicine market for a decade, primarily prescribed for the management of atopic dermatitis and allergic dermatitis causing pruritus in dogs aged 12 months or older. This medication acts as a selective Janus kinase (JAK) inhibitor, targeting essential cytokines in allergic responses, including IL-2, IL-4, IL-6, IL-13, and IL-31 (Gonzales et al. 2014). Cell-based *in vitro* studies have confirmed that oclacitinib does not significantly impact granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin, IL-12, or IL-23 at the concentrations tested (Gonzales et al. 2014)

The JAK-signal transducer and activator of transcription (STAT) pathway is vital in immune regulation, particularly concerning cytokine receptors and T helper cell polarization. CD4+ T helper cells can differentiate into various effector subsets, including Th1, Th2, Th17, and regulatory T cells (Seif et al. 2017). Notably, STAT3 is known to enhance the production of inflammatory cytokines, such as IL-17 and IL-6 (Kasmi et al. 2006).

Oclacitinib's efficacy is attributed to its selective inhibition of JAK1-dependent cytokines, demonstrating greater specificity compared to JAK2 or JAK3-dependent cytokines (Gonzales et al. 2014). JAK1 is involved in the signalling of 'γc' receptor cytokines (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21), pro-inflammatory cytokines including IL-6, as well as IFN. The critical position of JAK1 downstream of these cytokines suggests that JAK1-selective inhibitors are comparable to non-selective ones, without the unwanted consequences of JAK2- or JAK3-blockade. JAK1-selective drugs are also showing promise in human axial spondyloarthritis, suggesting that

they may target additional regulatory pathways that impact cytokines such as TNF and IL-17A, which do not use JAKs. Evidence now supports a JAK1 predominance in the signalling of IL-6 and oncostatin M, and indirectly, of TNF in synovial fibroblasts, macrophages and endothelial cells (Spinelli et al. 2021).

Currently, JAK inhibitors are approved for treating pruritus and atopic dermatitis in dogs, as well as myelofibrosis, rheumatoid arthritis, COVID-19, among other conditions in humans, as figure 3 shows.

Jakinib	Selectivity	Species	Approved indications
Oclacitinib	JAK1	Dog	Pruritus, atopic dermatitis
Ruxolitinib	JAK1, JAK2, JAK2V617F	Human	Myelofibrosis, polycythemia vera
Tofacitinib	JAK1, JAK2, JAK3	Human	Rheumatoid arthritis, PsA, UC, polyarticular course JIA
Baricitinib	JAK1, JAK2	Human	Rheumatoid arthritis, COVID-19 (emergency use authorization)
Upadacitinib	JAK 1	Human	Rheumatoid arthritis, PsA, atopic dermatitis, UC
Fedratinib	JAK2, JAK2V617F	Human	Myelofibrosis
Abrocitinib	JAK 1	Human	Atopic dermatitis
Delgocitinib	Pan JAK	Human	Atopic dermatitis
Figlotinib	JAK 1	Human	Rheumatoid arthritis
Pacritinib	JAK2, JAK2V617F	Human	Myelofibrosis
Peficitinib	Pan JAK	Human	Rheumatoid arthritis

JIA = Juvenile idiopathic arthritis. PsA = Psoriatic arthritis. UC = Ulcerative colitis.
JAK2V617F is an acquired, somatic mutation present in the majority of patients with myeloproliferative cancer.

Figure 3: Summary of approved Janus kinase (JAK) inhibitors. From: Marsella et al. 2023

For dogs, a daily oclacitinib dose effectively inhibits proinflammatory JAK1-dependent cytokines (IL-2, IL-4, IL-6, IL-13, IL-31), focusing its action on these cytokines over those not dependent on JAK1, which are vital for normal functions such as hematopoiesis. An alternative dosing regimen, involving administration every other day post-day 14, has been proposed to manage pruritus through day 30, offering a cost reduction strategy (Bizarro et al. 2022). Long-term oclacitinib administration is deemed safe and effective, significantly enhancing the quality of life. However, it has been observed that treated dogs exhibit decreased mean counts of white blood cells, neutrophils, monocytes, and eosinophils (Cosgrove et al. 2013).

Although oclacitinib is inhibited to be used in cats, in a study with pruritic cats oclacitinib was also shown to be well tolerated at 1 mg/kg and 2 mg/kg and appeared to be safe for this species when administered orally twice daily for 28 days (Cosgrove et al. 2013). Furthermore, in cats with experimental asthma, oclacitinib at 0.5 mg/kg or 1 mg/kg P.O. twice daily for 28 days significantly suppressed airway inflammation and eosinofilia and adverse clinical signs were not observed (Chang et al. 2013; Cosgrove et al. 2013; Mueller et al. 2021; Older et al. 2021).

Oclacitinib has also been explored off-label for managing pruritus in horses, addressing conditions such as insect bite hypersensitivity and atopic dermatitis (Marsella et al. 2023). A previous study was performed to characterize the pharmacokinetics and the clinical response

in horses using a single intravenous (n=4; 0,25 mg/kg IV) and oral dose (6; 0,2 mg/kg) and it showed that the half-life was identical for both routes and longer than dogs (7,5 to 8 hours) (Collard et al. 2020). The evaluation of adverse events and clinical pathology revealed no effects that appeared clinically significant or biologically important (Visser et al. 2020).

Given its ability to regulate neutrophil influx, oclacitinib presents a potential solution for asthmatic horses that fail to respond to glucocorticoids. By reducing neutrophil infiltration in the airways, oclacitinib may effectively decrease clinical signs associated with the condition.

Chapter 3. Objectives

It is known that some horses do not respond as expected to GC therapy. Those horses will live their lives with continuous signs of dyspnea. The motivation behind this study was to explore an alternative treatment that could improve the quality of life for these horses and their owners, offering an effective solution without using glucocorticoids.

In this pilot study we intended to evaluate the efficacy of a new treatment (Oclacitinib) in horses refered for glucocorticoid resistant EAS.

Research question (PICOT format): Is the oral administration of oclacitinib effective in the reduction of equine asthma clinical signs and BAL cytology, as compared to dexamethasone treatment, over a period of 6 months, in 6 privately owned horses diagnosed with equine asthma syndrome (EAS) by first opinion veterinarians and referred to the equine teaching hospital of FMV/ULisboa for glucocorticoid-resistant EAS?

Our research question is in agreement with the FINER criteria (Farrugia et al, 2010), and we suggested it based on personal experience, on knowledge from published papers, and on the recommendations made by other authors regarding further research suggestions. So, it's relevant.

Also, standard treatment (glucocorticoids), but not oclacitinib, is well known and included in the equine asthma syndrome consensus statement recommendations. It's also novel, as it will extend previous findings.

It's feasible, as EAS is a prevalent disease worldwide. The only compliance problem could be due to the need for long term treatment and to its cost therefore, we have scheduled monthly check visits by the investigators and asked for Zoetis sponsoring. Furthermore, we have the expertise for establishing and monitoring oclacitinib therapy in horses.

We aim to help horses and their owners, who deal daily with this disease, with repercussion on well-being, body condition and exercise performance of horses, implying also a financial loss. The effort to maintain compliance with the treatment for the length of six months aims

at creating a habit of pursuing with the treatment of these horses and reaching the time point where true positive changes can actually be acknowledged by both owners and veterinarians.

The research question suggested leads directly to hypotheses as to the predictions about the nature and direction of the relationship between the variables under study. We would like to hypothesise that the glucocorticoid resistant EAS affected horses taking oclacitinib (new treatment) will have a greater reduction in the outcomes studied, when compared to the horses taking dexamethasone (standard treatment).

Chapter 4. Materials and Methods

4.1 Horses

This study involved 6 privately owned horses between 11 and 20 years old, clinically diagnosed with EAS and referred by first opinion veterinarians to the Teaching Hospital of FMV/ULisboa for not responding to the 2 week standard treatment (dexamethasone). Inclusion criteria required horses to exhibit EAS clinical signs for at least one month despite 2 weeks standard glucocorticoid treatment. The horses were fully evaluated, following our referral asthma protocol, in order to be staged according to Tilley et al. (2012). Horses suffering from concurrent systemic disease or the ones that responded to glucocorticoid therapy in less than a month were excluded from the study. Consent was obtained from all owners for the participation of their horses in the study and an authorization from the Ethics Committee for Research and Education of FMV-ULisboa was conceded.

All horses performed a 2 week washout period between the standard treatment protocol and the new treatment protocol.

4.2 Protocols

4.2.1 Fluxogram

This was a study with a 2 week washout period, following the protocol explained on figure 4.

4.2.2 EAS staging

The EAS staging was performed on days 0 and 56 (last day of the New Treatment) at the Teaching Hospital- FMV ULisboa and was carried out according to Tilley et al. (2012), as is the normal protocol in our equine asthma referral consultation. This included a clinical score, a lung X-ray score, a respiratory endoscopy score and a BALF cytology score as can be seen in

figure 5.

Figure 4: Study protocol fluxogram

4.2.2.1 Clinical score (CS)

The clinical score is determined by assessing three parameters: cough, nostril flare and abdominal lift. The final partial score was calculated by summing the values from those three partial grades and it directly corresponds to the clinical score on a predefined scale.

The clinical score was obtained on days 0 and 15 of the Standard Treatment (ST), on days 0, 28, 56 of the New Treatment (NT) and on day 180 at the end of the 4 months follow up period from the New Treatment, in order to determine the evolution of clinical signs before, during and after both treatments.

4.2.2.2 Endoscopic score (ES)

The endoscopic score was determined by evaluating four parameters: mucus accumulation, mucus color, mucus localization, and mucus stickiness and apparent viscosity. The sum of those grades produces the final partial score, which corresponds directly to the endoscopic score on a predefined scale. The endoscopic score was assessed on days 0 and 56 using nasobronchial endoscopy at the Teaching Hospital of FMV ULisboa. The images were subsequently reviewed by two independent clinicians, following the criteria outlined by Tilley et al. (2012), to assign individual scores for each parameter.

Table 1
Clinical staging of RAO sheet (only selected variables with correlation coefficients higher than 0.60 are considered[a]).

GRADE	0	1	2	3	4	5
CS						
Cough Score ☐	None	Coughs on specific times of day (feeding/ exercising/making beds)	Frequent cough with periods of no coughing	Very frequent cough		
Nostril flare score[b] ☐	None	Flares during inspiration (returns to normal at end inspiration)	Flares in inspiration and exhalation (slight movement can still be seen)	Flares in inspiration and expiration (no movement can be seen)		
Abdominal lift score[b] ☐	None	Slight flattening of ventral flank	Obvious abdominal flattening and "heave line" extending no more than half way between cubital joint and *tuber coxae*	Obvious abdominal lift and "heave line" extending beyond halfway between cubital joint and *tuber coxae*		
CS final score = Cough score + Nostril flare score + Abdominal lift score =						
Score: 0 (CS final score < 2)/ **1** (2 ⩽ CS final score ⩽ 4)/ **2** (5 ⩽ CS final score ⩽ 6)/ **3** (7 ⩽ CS final score ⩽ 9) ☐						
ES[c]						
Mucus accumulation ☐	None, clean	Little, multiple small blobs	Moderate, larger blobs	Marked, confluent or stream-forming	Large, pool-forming	Extreme, profuse amounts
Mucus colour ☐	None, clean	Colourless	White	Thick White	Yellow	Thick Yellow
Mucus localization and stickiness ☐	None, Clean	1/2 Ventral	2/3 Lateral	3/4 Dorsal	Threading	Threading
Mucus apparent viscosity ☐	None, Clean	Very fluid	Fluid	Intermediate	Viscous	Very viscous
ES final score = Mucus accumulation + Mucus colour + Mucus localization and stickiness + Mucous apparent viscosity =						
Score: 0 (ES final score < 8.5)/ **1** (8.5 ⩽ ES final score ⩽ 12)/ **2** (12 < ES final score ⩽ 16)/ **3** (ES final score > 16) ☐						
BALFS						
Neutrophil% (400 nucleated cell count) ☐	⩽20	21–40	41–60	⩾61		
BALFS final score = Neutrophil% =						
Score: 0 (BALFS final score ⩽ 20)/ **1** (21 ⩽ BALFS final score ⩽ 40)/ **2** (41 ⩽ BALFS final score ⩽ 60)/ **3** (BALFS final score ⩾ 61) ☐						
XRS						
Interstitial pattern ☐	No parenchial opacity	Mild increase of parenchial opacity	Moderate increase of parenchial opacity	Increased parenchial opacity with loss of vizualization of vascular structures		
Bronchial radiopacity ☐	Normal	Mild increase	Moderate increase	Severe increase		
Tracheal thickening ☐	No increase in thickening	Mild increase in thickening	Moderate increase in thickening	Severe increase in thickening		
Bronchial thickening ☐	No increase in thickening	Mild increase in thickening	Moderate increase in thickening	Severe increase in thickening		
XRS final score = Interstitial pattern + Bronchial radiopacity + tracheal thickening + bronchial thickening =						
Score: 0 (XRS final score < 5)/ **1** (5 ⩽ XRS final score ⩽ 6)/ **2** (6 <XRS final score ⩽ 8)/ **3** (XRS final score > 8) ☐						
Total Score = CS final score + ES final score + BALFS final score + XRS final score =						
RAO Stage =	Stage 0 – No RAO (Total Score < 5) ☐	Stage 1 – Latent RAO (5 ⩽ Total Score ⩽ 7) ☐	Stage 2 – Mild RAO (8 ⩽ Total Score ⩽ 9) ☐	Stage 3 – Moderate RAO (10 ⩽ Total Score ⩽ 11) ☐	Stage 4 – Severe RAO (Total Score ⩾ 12) ☐	

[a] All grading systems suggested comprise four grades, except for endoscopic grading which was adapted from previously published data where six grades were considered.
[b] Adapted from Gerber et al. (2000).
[c] Adapted from Gerber et al. (2004).

Figure 5: EAS staging protocol. From: (Tilley et al. 2012)

4.2.2.3 BALF score

The BALF score was determined by the percentage of neutrophils in a BALF cytology sample obtained via a nasobronchial BAL tube. This score was measured on days 0 and 56 at Teaching Hospital- FMV ULisboa . The samples were collected into 10 mL potassium-EDTA tubes, refrigerated, and sent to DNAtech Laboratory for cytological analysis.

4.2.2.4 X-ray score (XRS)

The X-ray score was calculated by evaluating four specific parameters: interstitial pattern, bronchial radiopacity, tracheal thickening, and bronchial thickening. The combined total of these individual grades results in the final partial score, which directly corresponds to the X-ray score on a standardized scale. X-ray score was conducted on days 0 and 56 at the Teaching Hospital of FMV ULisboa. Lung X-rays were captured at the end of inspiration with exposure settings of 100 kV and 25-32 mA. The resulting images were reviewed by two independent clinicians based on the guidelines provided by Tilley et al. (2012) to determine the individual scores for each parameter.

4.2.2.5 Total Score (TS) and Stage

The total score was determined by summing the four scores mentioned above. This total score directly corresponds to a specific EAS stage on a standardized scale, allowing for an accurate diagnosis of the horse's condition, as outlined by Tilley et al. (2012).

4.2.3 Leukogram

Samples were collected from each horse, from the jugular vein into a 10 ml tube containing potassium-EDTA. The tubes were immediately refrigerated and sent to Urano Lab to conduct a leukogram. Samples were collected on day 0, 56 and 180, in order to evaluate the influence of oclacitinib in circulating neutrophils percentage before, during and after treatment.

4.2.4 Standard treatment administration (Dexamethasone)

According to Mainguy-Seers and Lavoie (2021) the standard treatment protocol with dexamethasone for 2 weeks was performed as follows:

- 0.06 mg/kg IV q.24h for 5 days
- 0.04 mg/kg IV q.24h for 2 days
- 0.04 mg/kg IV q.48h for 7 days

4.2.5 New treatment administration (Oclacitinib)

The new treatment protocol using oclacitinib was as follows:

- Started Oclacitinib (Apoquel 16mg tablets, Zoetis) at 0.25 mg/kg per os q.24 hours, which means 7 tablets/day for an average 450kg horse, based on Visser at al. (2020) and Marsella et al. (2023) treatment protocol for equine allergic dermititis.
- If asthma clinical signs were in remission after 28 days the dose of Oclacitinib was reduced to 0.25 mg/kg per os on alternate days for a further 28 days to reduce costs, according to the suggestion by Bizarro et al (2022) for use in dogs. Otherwise, the once a day administration was maintained.
- Horses were followed for another 4 months after the end of treatment.

4.2.6 Monthly visits

All horses were visited monthly and a clinical score (CS), identical to the one carried out for the staging of equine asthma, was attributed. Horse owners were also asked to answer some questions about the medication being administered to their horse. These visits also served to supervise and register owner/carer compliance. Any existing doubts were clarified during these visits.

4.2.7 Statistical Analysis

Descriptive statistics were employed to analyze the data collected from the study. The mean and standard deviation (SD) were calculated for each variable (SC, ES, BALFS, XRS, TS, leukogram) in all time points to summarize the central tendency and dispersion of the data. Percentage differences were also computed to assess the relative change in specific parameters over time, as presented in the equation 1, in which the original value corresponds to the one on day 0 of the New Treatment and the final value is referred to the one in which the evaluation was preformed. For missing data, values were imputed using the mean of the corresponding measurements from the other subjects. These statistical measures were utilized to evaluate the progression of clinical, endoscopic, BALF, and X-ray scores across different time points (days 0, 28, 56, and 180) in order to access the effectiveness of the treatment.

$$Percentage\ difference = \frac{final\ value - original\ value}{original\ value} \times 100 \quad (1)$$

Chapter 5. Results

Since H4 did not finish the treatment, in order to be included in the study, the results of day 56 and 180 for this horse were estimated using the mean of the other five horses, as explained previously in section 3.2.5. This missing data is missing at random and represents a low percentage of the total data, therefore it is useful to use an imputation method rather than removing the data. In this case, for H4 we imputed the mean of the existing observations and inserted it in the place of the missing observations.

5.1 EAS staging

Recalling the section 3.2 of Material and Methods, the following results were obtained according to Tilley et al (2012) EAS staging protocol.

5.1.1 Clinical Score (CS)

Figure 6 illustrates the progression of the final partial clinical scores for the six horses at days 0, 28, 56 (last day of treatment), and 180 of the new treament with oclacitinib.

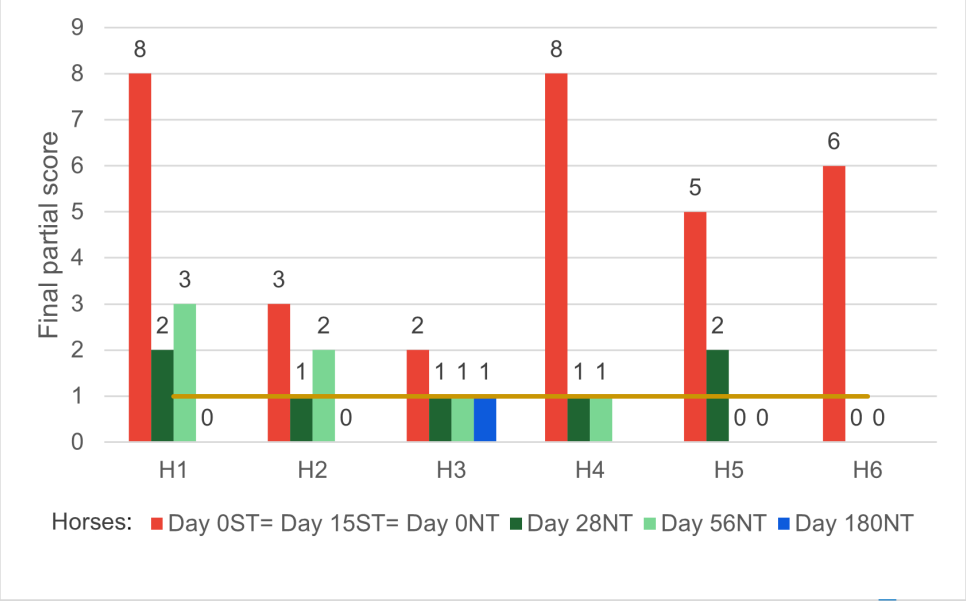

Figure 6: Final partial scores score, on days 0 and 15 of the Standard Treatment, and on days 0,28,56,180 of the New Treatment

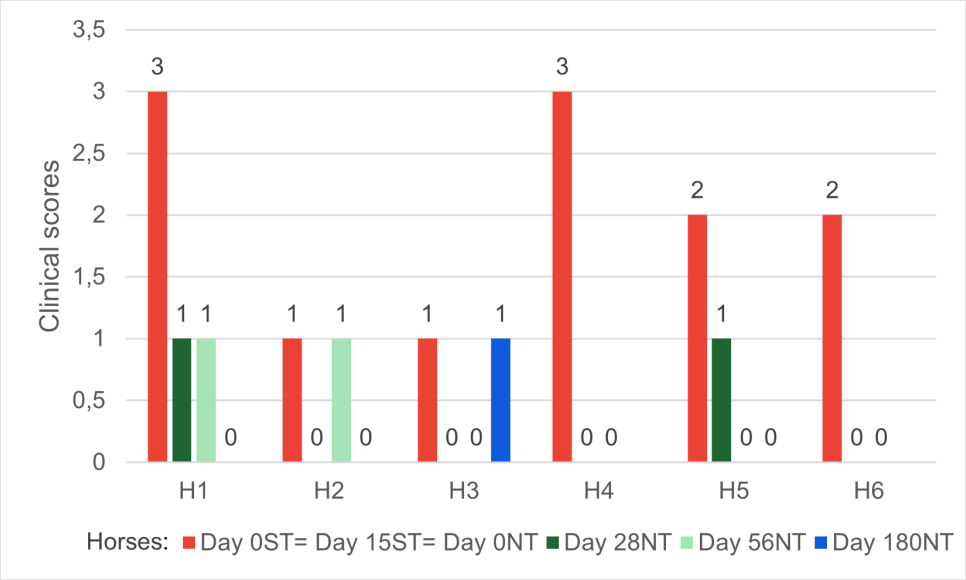

Figure 7: Clinical Scores on days days 0 and 15 of the Standard Treatment and on days 0,28,56,180 of the New Treatment

Two-thirds of the horses (H3, H4, H5 and H6) ended the new treatment with a final partial score ≤ 1, meaning a clinical score of 0. H1 and H2 ended with a final partial score ≥ 2 and ≤ 4, therefore a clinical score of 1.

Figure 7 summarizes the clinical scores which were obtained on a predefined scale based on the final partial scores on each day that were presented in figure 6 (Tilley et al. 2012).

Clinical score - Day 0 Standard Treatment (ST) and Day 15 ST

The clinical scores recorded on the first and last days of the standard treatment with dexamethasone showed no substantial differences between them, ranging from 3 to 1 on both day 0 and day 15. These scores were consistent with those observed on day 0 of the new treatment with oclacitinib.

Clinical score - Day 0 New Treatment (NT)

The highest final partial score on day 0 was 8, which was obtained for H1 and H4, while the lowest final partial score was 2, recorded for H3. Therefore, the clinical scores on day 0 ranged between 3 and 1.

Clinical score - Day 28 NT

On day 28, the final partial scores ranged between 2 and 1 therefore the clinical scores were between 1 and 0.

After 28 days of daily oclacitinib treatment, there was an average improvement of 83% with reference to day 0. Four of the six horses (H2, H3, H4 and H6), achieved a clinical score of 0 presenting a 100% improvement, while H5 exhibited a 50% improvement and H1 a 67% improvement, having both reached a clinical score of 1.

Clinical score - Day 56 NT

On day 56, the last day of treatment, the final partial scores ranged between 3 and 0. Accordingly, the interval of clinical scores was between 1 and 0.

After 56 days of oclacitinib treatment (28 days daily followed by 28 days on alternate days), there was an average improvement of 83% with reference to day 0. H2 did not show any improvement, maintaining a score of 1. H1 improved by 67%, while H3, H4, H5, and H6 showed a 100% improvement.

Furthermore, two-thirds of the horses showed no differences when switched to alternate days (H1, H3, H4 and H6), while H2 showed better improvements on day 28 and H5 showed a progressive improvement during the treatment.

Clinical score - 180 days NT

On day 180, the final partial scores ranged between 1 and 0 and consequently the interval of clinical scores was between 1 and 0.

After 180 days (56 days of oclacitinib treatment followed by 124 days without treatment), H1, H2 and H5 showed a 100% improvement compared to the beginning of treatment, while H3 regressed to a score of 1, the same as at day 0.

5.1.2 Endoscopic Score (ES)

Figure 8 presents the progression of the endoscopic final partial scores for the six horses on days 0 and 56 of the new treatment.

Figure 8 shows that on day 0, the final partial scores ranged between 13 and 9 and consequently the interval of endoscopic scores was between 2 and 1, while on day 56 all horses presented an endoscopic final partial score ≤ 8, concluding that all horses ended the treatment with an endoscopic score of 0.

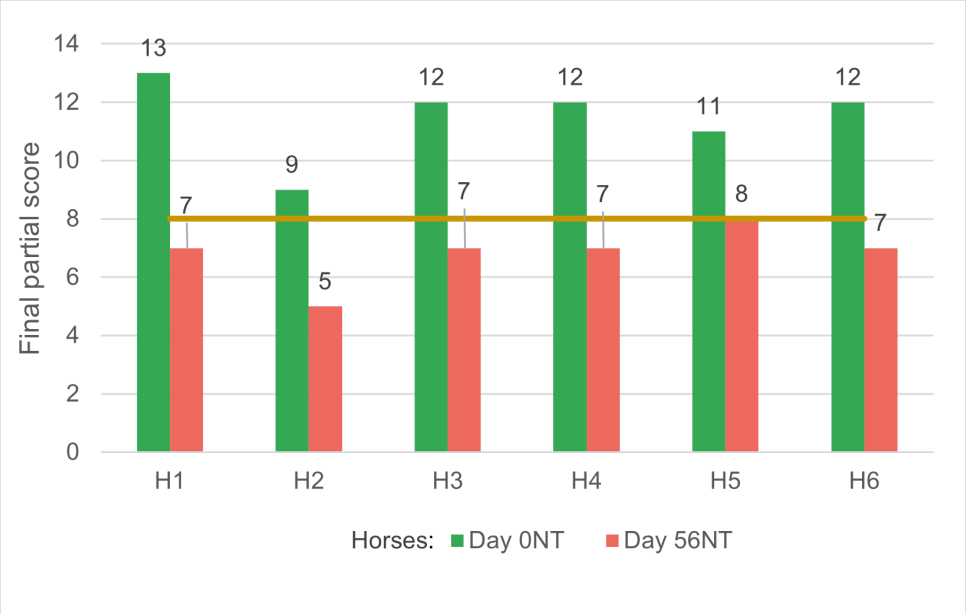

Figure 8: Final partial score for ES, on days 0,28,56,180 of the New Treatment

After 56 days of the new treatment, there was an average improvement of 100%, with all horses achieving a score of 0. Among the variables studied, mucus accumulation showed the most significant reduction (30%), while mucus color showed no change. Mucus localization and stickiness and apparent viscosity showed a 27% decrease. Table 3 summarizes the improvements in the four variables studied, from day 0 to day 56 of the new treatment.

Improvement - ES		H1	H2	H3	H4	H5	H6	Average	Standard deviation
Day 0	Mucus accumulation	3,00	3,00	4,00	5,00	5,00	4,00	4,00	0,82
	Mucus colour	2,00	2,00	2,00	2,00	2,00	3,00	2,17	0,37
	Mucus localization and stickiness	1,00	1,00	3,00	2,00	2,00	2,00	1,83	0,69
	Mucus apparent viscosity	1,00	3,00	3,00	3,00	2,00	3,00	2,50	0,76
Day 56	Mucus accumulation	4,00	1,00	3,00	3,00	3,00	2,00	2,67	0,94
	Mucus colour	3,00	2,00	2,00	2,00	2,00	2,00	2,17	0,37
	Mucus localization and stickiness	3,00	1,00	1,00	1,00	1,00	1,00	1,33	0,75
	Mucus apparent viscosity	3,00	1,00	1,00	2,00	2,00	2,00	1,83	0,69

Average Improvement	Mucus accumulation	33%
	Mucus colour	0%
	Mucus localization and stickiness	27%
	Mucus apparent viscosity	27%

Table 3: Improvement of the four parameters evaluated for the Endoscopic Score on days 0 and 56 of the New Treatment

5.1.3 BALF Score

Figure 9 displays the changes in the percentage of neutrophils in bronchoalveolar lavage fluid (BALF) on days 0 and 56 of the new treatment.

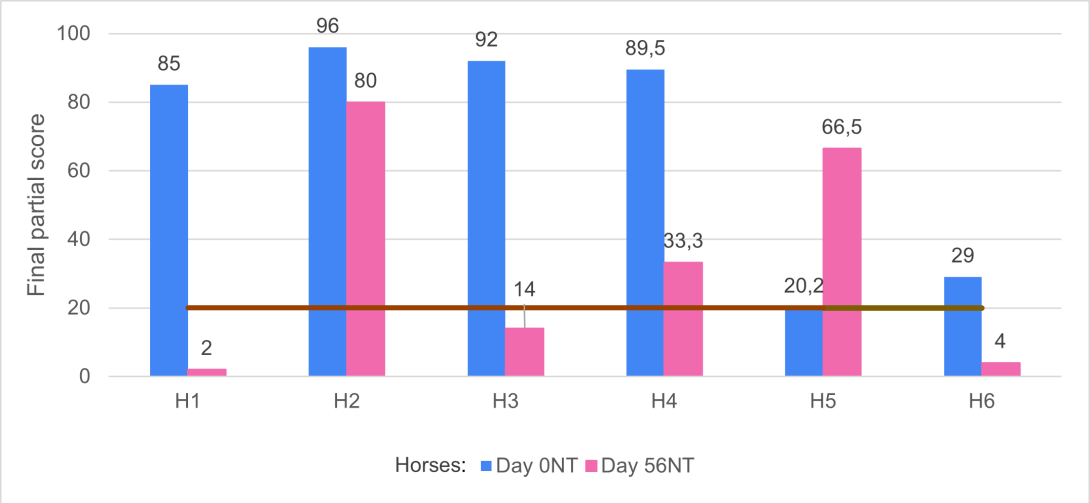

Figure 9: Final partial scores for BALFS, on days 0,28,56,180 of the New Treatment

On day 56, there was an average 46% improvement in the BALF score. H2 retained its initial score of 3, although there was a small decrease in neutrophils percentage, while H5 worsened from stage 1 to stage 3. H1, H3 and H6 showed a 100% improvement, and H4 had an estimated virtual result of 67%.

Besides, figure 9 presents that half of the horses (H1, H3 and H6) ended the new treatment with a BALF neutrophil percentage $\leq$ 20% which means these horses on day 56 presented a BALF score of 0 whereas H4 ended with a BALF score of 1 ($\geq$ 21% and $\leq$ 40%) and H2 together with H5 with a BALF score of 3 (neutrophil percentage $\geq$ 61%)

5.1.4 X-ray Score (XRS)

Figure 10 presents the progression of the X-ray final partial scores for the six horses on days 0 and 56 of the new treatment.

Figure 10 indicates that a third of the horses (H3 and H6) ended the treatment with a XRS final partial score $\leq$ 4, concluding these two horses on day 56 presented a X-ray score of 0. H2 along with H4 and H5 ended with a final partial score $\geq$ 5 and $\leq$ 6, representing an X-ray score of 1, while H1 ended with a final partial score $\geq$ 7 and $\leq$ 8, therefore a an X-ray score of 2.

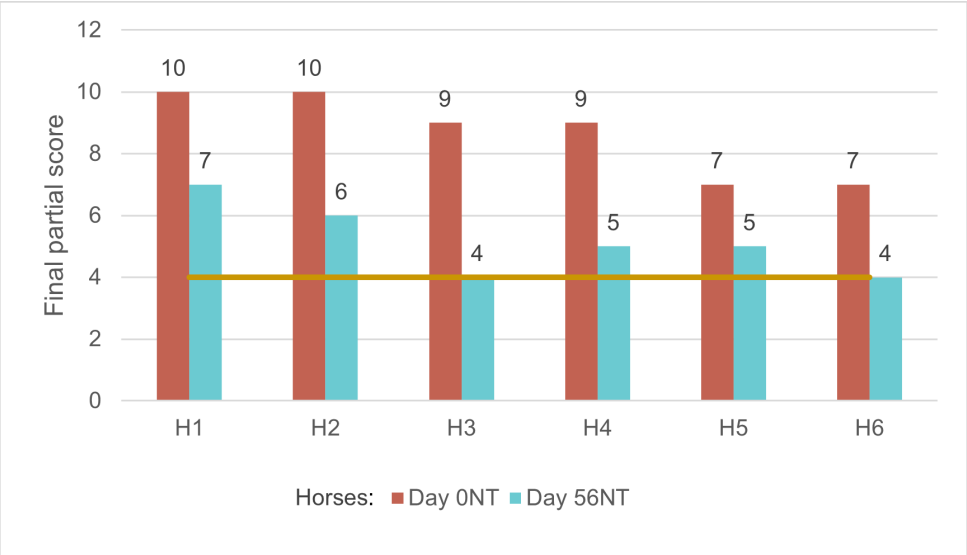

Figure 10: Final partial scores for XRS, on days 0,28,56,180 of the New Treatment: interstitial pattern, bronchial radiopacity, tracheal thickening and bronchial thickening grades

On day 56, the average X-ray score improvement was 75%. H3 and H6 showed a 100% improvement. H1 had the lowest improvement at 33%, while H5 improved 50% and H2 and H4, 67%. Among the variables studied, the interstitial pattern showed the most significant change (50%), while tracheal thickening showed no improvement. Bronchial thickening improved by 47%, and bronchial radiopacity by 31%. Table 4 summarizes the improvements in the four variables studied, based on the grades obtained on day 0 and 56.

Improvement - XRS		H1	H2	H3	H4	H5	H6	Average	Standard Deviation
Day 0	Interstitial pattern	2,00	2,00	1,00	1,00	2,00	2,00	1,67	0,47
	Bronchial Radiopacity	3,00	3,00	3,00	3,00	2,00	2,00	2,67	0,47
	Tracheal thickening	0,00	2,00	2,00	2,00	1,00	1,00	1,33	0,75
	Bronchial thickening	2,00	3,00	3,00	3,00	2,00	2,00	2,50	0,50
Day 56	Interstitial pattern	2,00	2,00	1,00	1,00	1,00	1,00	1,33	0,47
	Bronchial Radiopacity	3,00	2,00	1,00	2,00	2,00	1,00	1,83	0,69
	Tracheal thickening	3,00	1,00	1,00	1,00	1,00	1,00	1,33	0,75
	Bronchial thickening	3,00	1,00	1,00	1,00	1,00	1,00	1,33	0,75

Average Improvement	Interstitial pattern	50%
	Bronchial Radiopacity	31%
	Tracheal thickening	0%
	Bronchial thickening	47%

Table 4: Improvement of the four parameters evaluated for the X-ray Score on days 0 and 56 of the New Treatment

5.1.5 Total Score and Asthma Staging

The total score, summing the four parameters mentioned above, showed that all horses showed an overall improvement: H3 and H6 had a 100% improvement post-treatment, while H4 and H5 showed the lowest improvement at 20%. H1 improved by 73%, and H2 by 38%.

The total score allows for the staging of Equine Asthma Syndrome. At the beginning of the new treatment, H4 was diagnosed with stage 3, H1, H2, and H3 were at stage 2, and H5 and H6 were at stage 1. By the end of the treatment, all horses except H2 achieved stage 0, indicating the absence of disease. It should be noted however that H2 showed improvement, reaching stage 1.

Figures 11 and 12 confers the progression of the total scores and corresponding stages of EAS, respectively, for the six horses at the beginning and the end of the new treatment.

Figure 11: Total score for all horses on days 0 and 56 of the New Treatment

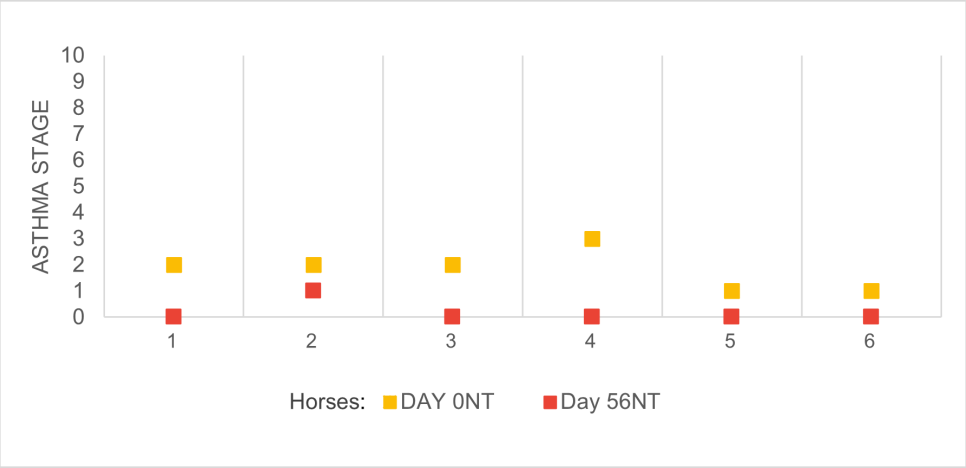

Figure 12: Asthma Stage for all horses on days 0 and 56 of the New Treatment

5.2 Leukogram

On day 0, all horses had leukocyte counts within the normal range of values established by the laboratory, but H1, H3, H4, and H5 had neutrophil counts above normal. H2 and H6 were within the normal range established by the laboratory.

On day 56, all horses had leukocyte counts within the normal range and only H6 had a slight deviation in neutrophil count ($0.14 \times 10^3\ \mu l$ above normal).

On day 180, H1, H2 and H5 remained within the normal range for both leukocyte and neutrophil counts, while H3 showed an elevated neutrophil count.

Table 5 summarizes the counting of leukocytes and neutrophils on days 0, 56 and 180. The results above the normal range are highlighted.

Leukogram		H1	H2	H3	H4	H5	H6	Reference values for Urano Lab
Day 0	Leukocytes (x10ˆ3/l)	10,94	9,69	13,15	13,82	10,45	8,58	5,2-13,9
	Neutrophils (x10ˆ3/l)	9,24	6,05	8,62	10,44	7,48	6,16	2,7-6,7
Day 56	Leukocytes (x10ˆ3/l)	8,41	9,00	12,70	9,65	8,89	9,26	5,2-13,9
	Neutrophils (x10ˆ3/l)	6,15	3,90	6,00	6,15	5,84	6,84	2,7-6,7
Day 180	Leukocytes (x10ˆ3/l)	8,31	12,37	11,60		8,85		5,2-13,9
	Neutrophils (x10ˆ3/l)	4,39	6,10	7,80		4,72		2,7-6,7

Table 5: Leukocytes and Neutrophils counts on days 0, 56 and 180 of the New Treatment

Chapter 6. Discussion

6.1 EAS staging

6.1.1 Clinical score (CS)

The clinical score allows us to evaluate the evolution of clinical signs in this 6 months timeline.

Clinical score - Day 0 ST and Day 15 ST

The clinical scores recorded on the first and last days of the standard treatment with dexamethasone showed no substantial differences between them, as expected since all horses were referred as non-responsive to glucocorticoids, therefore no improvement was expected from this therapeutic.

Clinical score - Day 28 NT

After 28 days of daily treatment with oclacitinib, all horses exhibited improvement in clinical signs. H2, H3, H4, and H6 achieved a score of 0, while H1 and H5 decreased to a score of 1. This discrepancy could be attributed to the lack of environmental control observed during the visit to H1, where the horse was stabled in a poorly ventilated indoor stall, increasing the chances of antigen exposure. In contrast, the other four horses were kept outside in paddocks with better ventilation. In the case of H5, although the horse was kept outside, some clinical signs persisted, likely due to a dust cloud from North Africa that affected air quality and

increased antigen exposure in the week of the visit.

The results obtained up to this point indicate a positive effect of daily oclacitinib administration at a dosage of 0.25 mg/kg per os on clinical signs, with no reported side effects. The findings also underscore the importance of environmental control, particularly adequate ventilation, in reducing antigen exposure (Simões et al. 2020) (Mainguy-Seers and Lavoie 2021).

Clinical score - Day 56 NT

On day 56 of the new treatment, two-thirds of the horses (H3, H4, H5, and H6) achieved a clinical score of 0, while H1 and H2 had a clinical score of 1. Notably, H2's condition slightly worsened when oclacitinib was administered on alternate days. Studies including larger sample sizes are needed to confirm the need to maintain the everyday administration of oclacitinib. Even though reducing oclacitinib administration after day 28 to every other day is economically desirable, it is possible that some horses would benefit from daily medication for a longer period of time. For H1, external factors such as owner inefficient compliance and incorrect administration techniques may have contributed to the lack of improvement between days 28 and 56.

Overall, it can be concluded that two-thirds of the horses showed no clinical differences when switched to alternate-day dosing (H1, H3, H4, and H6), while H2 exhibited better improvement on day 28, and H5 showed continuous improvement throughout the treatment. Thus, on average, there appears to be no noteworthy difference in clinical outcomes between daily administration and alternate-day dosing of oclacitinib at 0.25 mg/kg per os.

Clinical score - Day 180 NT

After 180 days (comprising 56 days of treatment followed by 124 days without treatment) H1, H2 and H5 exhibited a 100% improvement compared to their scores at the beginning of treatment. In contrast, H3 regressed to a score of 1, identical to its score on day 0. This regression may be linked to a secondary respiratory infection that began 10 days prior to the evaluation, resulting in inspiratory dyspnea, nasal discharge, and reduced appetite.

Based on these results we can conclude that in this horse sample, oclacitinib was effective in controlling clinical signs and avoid clinical exacerbation of EAS, when associated with environment control.

6.1.2 Endoscopic Score

The Endoscopic Score (ES) evaluates the progression of four parameters visualized through nasobronchial endoscopy: mucus accumulation, mucus colour, mucus localization, and stickiness, as well as apparent viscosity. The ES was assessed on days 0 and 56.

On day 0, all horses had an ES $\geq$ 1. The highest sum of partial scores was observed in H1 (13), while H2 had the lowest one (9). As shown in table 3 in 5.1.2 the parameter with the highest grades was mucus accumulation, with H4 and H5 both reaching the maximum grade of 5. Mucus localization and stickiness exhibited the lowest grades, with an average of 1.83 ± 0.69.

By day 56, all horses achieved an ES of 0. Mucus accumulation remained the parameter with the highest grades, averaging 2.67 ± 0.94. Notably, this parameter showed the greatest improvement, with a 33% reduction. Mucus localization and stickiness again had the lowest grades, averaging 1.33 ± 0.75, reflecting a 27% improvement, along with mucus apparent viscosity. Mucus color, on average, did not show improvement during the 56 days of treatment.

These findings indicate that this protocol of treatment with oclacitinib effectively reduced the ES to 0 in all six horses within 56 days, with the biggest impact on mucus accumulation. This outcome is likely due to oclacitinib's inhibitory effect on inflammation, selectively targeting JAK-1-dependent cytokines (Gonzales et al. 2014).

6.1.3 BALF score

The BALF score (BALFS) assesses the percentage of neutrophils present in bronchoalveolar lavage fluid (BALF). The BALFS was measured on days 0 and 56. Neutrophils are the predominant cells in the bronchoalveolar fluid of asthmatic horses (Nocker et al. 1996; Medoff et al. 2002; Singh et al. 2014; Molet et al. 2001), making their percentage a key indicator for this score.

On day 0 of the new treatment H1, H2, H3, and H4 exhibited very high neutrophil percentages, ranging from 96% to 89.5%, resulting in a BALFS of 3, which aligned with the clinical signs. H6 and H5 showed a lower percentage of 29% and 20,2%, respectively, corresponding to a score of 1.

By day 56, half of the horses (H1, H3, and H6) achieved a BALFS of 0, while H2's score remained unchanged. H3 and H6 demonstrated a 100% improvement, whereas H5 showed an unexpected increase in neutrophil percentage, with no clear explanation identified and no concurrent increase of any other variable. H4 exhibited a 67% improvement.

These findings suggest that the oclacitinib treatment protocol was effective in reducing neu-

trophil counts in 4 out of 6 horses. Extending the daily treatment duration or increasing the dosage could be strategies for improving responses in the horses that did not meet expectations.

Oclacitinib is a Janus kinase inhibitor that selectively targets JAK1-dependent cytokines. Several cytokines involved in allergic conditions require JAK1 for activation. For instance, IL-6, produced by various cells such as T cells and macrophages, plays a crucial role in innate immune activation, including neutrophil migration. IL-2, another JAK1-dependent cytokine, regulates T-cell proliferation and can induce the production of T-cell cytokines like IFN-γ (Gonzales et al. 2014). Given Oclacitinib's inhibitory effects, a reduction in airway neutrophils is anticipated. On average, a 46% improvement in the BALF score was observed. However, due to the small sample size, these findings cannot be generalized to the broader population.

6.1.4 X-ray Score

The X-ray Score (XRS) assesses the progression of four parameters evaluated through lung X-ray: interstitial pattern, bronchial radiopacity, tracheal thickening, and bronchial thickening. The XRS was measured on days 0 and 56.

On day 0, all horses had an XRS $\geq$ 2. H1 and H2 had the highest final scores (10), which may be attributed to the chronic nature of their condition, as these were the oldest horses in the sample, both 19 years old. As detailed in table 4 found in 5.1.4, bronchial radiopacity was the parameter with the highest grades, with H1, H2, H3, and H4 all receiving the maximum grade of 3. Tracheal thickening had the lowest grades, on average 1.33 ± 0.75.

By day 56, only H3 and H6 achieved an XRS of 0. The remaining horses showed improvements but did not reach stage 0. Despite the chronic effects, H2 showed a 67% improvement, as did H4, while H1 improved by 33%. Bronchial radiopacity had an improvement of 31% and remained the parameter with the highest grades, with an average score of 1.83 ± 0.69, while the other three parameters showed similar averages (1.34). Interstitial pattern presented the greatest improvement of 50%, along with bronchial thickening which improved in 47%. Tracheal thickening didn't show any improvement during the 56 days of treatment.

According to these results, this protocol with oclacitinib was only able to reduce the XRS to 0 in one-third of the sample. However, improvements between 33 an 67% were observed in the remaining horses, indicating a positive but limited effect within the 56-day treatment period, regarding these more chronic changes.

6.1.5 Total Score and Asthma staging

The total score represents the sum of the four parameters previously mentioned and provides an overall assessment of the horse's condition. On average, there was a 71% improvement in the total score across this sample of horses with the new treatment. H3 and H6 demonstrated the most significant improvement, achieving a 100% reduction in their scores. H1 showed a more modest improvement of 63%, likely due to delayed environmental control measures to reduce allergen exposure, as well as the horse's advanced age. Similarly, H2, also 19 years old, exhibited only a 38% improvement, which may be attributed to the chronic nature of its condition, making regression more difficult. H4 and H5 showed a 20% improvement; in H4's case, a more substantial improvement was anticipated if the protocol had been completed, as positive outcomes were observed on day 28. For H5, the lower-than-expected percentage of neutrophils on day 0 and their increase on day 56 contributed to the smaller improvement, although the horse showed complete clinical recovery.

Regarding asthma staging, the results were promising, with only H2 failing to achieve stage 0 by the end of the new treatment (day 56). This was likely due to the lack of improvement of the clinical score and of the BALF score on day 56, possibly linked to the respiratory infection that began on day 42, which may have influenced the outcomes expected on day 56. Nevertheless, given its age and other individual factors, this horse may have benefited from an extended daily treatment period or a higher dosage of the medication to achieve stage 0.

The results obtained in this group of horses corroborate our initial hypothesis that this protocol with oclacitinib would be effective in the control of glucocorticoid resistant asthma. This can be a relevant contribute to these horse's quality of life and to their enhanced performance.

6.2 Leukogram

Leukogram analyses were conducted on days 0, 56, and 180 to evaluate whether the oclacitinib treatment protocol adversely affected circulating white blood cells, particularly neutrophils. Notably, two-thirds of the sample (H1, H3, H4, and H5) exhibited an increased percentage of circulating neutrophils on day 0, consistent with Barton and Gehlen (2016), who reported that elevated peripheral blood leukocytes and circulating inflammatory mediators are observed during disease exacerbation, suggesting that the inflammatory process may extend beyond the lungs (Barton and Gehlen 2016). By day 56, neutrophils levels of all horses had decreased to within the standard range established by the laboratory. By day 180, only H3 exhibited an increase in circulating neutrophils, likely related to ongoing airway inflammation. Leukocyte counts remained within the normal range throughout the study.

These results suggest that the oclacitinib treatment protocol did not negatively impact the production of circulating leukocytes, including neutrophils, and was effective in normalizing neutrophil levels when elevated in cases of disease exacerbation.

Chapter 7. Conclusion

Equine Asthma Syndrome represents a prevalent chronic respiratory condition which has significant impact in the life of the horses.

Despite its impact, there's still no effective treatment for this condition. Although glucocorticoids most of the times can improve clinical signs within days, they offer limited benefits after treatment cessation. Moreover, some asthmatic horses present insensitivity to glucocorticoids, leaving no anti-inflammatory treatment for these cases.

This was the first study performed to evaluate the use of oclacitinib in the treatment of equine asthma patients which are non-responsive to glucocorticoids.

The results obtained for this group of horses were promising. There were improvements in almost all the scores which were evaluated: clinical score, endoscopic score, BALF score and total score. Only the X-ray score did not exhibit the anticipated level of improvement, though positive changes were still observed. The endoscopic score showed the most substantial progress, with an average 100% improvement by day 56. Moreover, the results obtained on day 180 (after 56 days of treatment followed by 124 days without treatment) for clinical signs were notably positive.

Regarding asthma staging, only one horse concluded the treatment with a clinical score of 1, while the remaining five horses achieved a score of 0, indicating the absence of disease.

Additionally, the study underscored the critical importance of environmental control in reducing antigen exposure. Alongside pharmacological treatment, this approach is essential for managing the condition and preventing exacerbations.

Over the six-month follow-up period, no adverse changes in leukocyte levels, particularly neutrophils, were observed. Leukocyte counts remained within the normal range throughout the study, and oclacitinib effectively normalized elevated neutrophil levels during the episode of disease exacerbation.

No side effects were detected during the entire study, though further research is necessary to confirm the long-term safety of this treatment.

Unexpected outcomes could be explained by potential issues in data collection and analysis, particular horse characteristics (e.g., age) and concurrent diseases, suboptimal owner compliance (e.g.,defaults of administration, lack of environment control) and even improper dosage or

duration of treatment.

To enhance the effectiveness of the oclacitinib treatment, several strategies could be considered: modifying the treatment protocol to involve higher doses or extending the duration of daily treatment; working with the owners over the importance of the adherence to treatment and environmental control measures (e.g., keeping horses outdoors, wetting straw, and avoiding dusty environments); ensuring consistency in data collection and analysis. Lastly, pharmaceutical innovations such as creating a more suitable formulation for horses (e.g., a powdered form with higher doses of oclacitinib) could further improve treatment outcomes.

In conclusion, the primary objective of assessing the efficacy of oclacitinib in treating steroid-resistant asthma phenotypes to control inflammation, alleviate clinical signs, and manage asthma exacerbations was achieved in this group of horses. This treatment has the potential to benefit horses and their owners, improving well-being, body condition, and performance, thereby restoring the quality of life that these animals deserve.

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Chapter A. Appendix

A.1 Appendix 1

Figure 13: Poster for the Scientific Interaction Project

A.2 Appendix 2

1- Programa de inteligência artificial para o diagnóstico e estadiamento da asma no cavalo - Zoom de França (Paris)

(Carolina Gomes - tese conjunta entre a Faculdade de Medicina Veterinária e o Departamento de Engenharia Informática do Instituto Superior Técnico)

2- Validação de dois métodos de estadiamento da asma no cavalo por comparação com "Goldstandards"

(Sara Santos – Tese em colaboração com a Universidade de Berna-Suiça)

3- Avaliação clínica de uma nova terapêutica para a asma no cavalo

(Mariana Ferreira – tese em colaboração com a empresa farmacêutica da área do medicamento)

4- Sensibilizações a alergenos de origem animal (incluindo o Homem) em cavalos do Ribatejo e Oeste

(Tânia Reis – tese em colaboração com uma empresa farmacêutica da área de alergologia)

5- Utilização do "Australian Noseband" para prevenir a obstrução respiratória durante o exercício no cavalo – Zoom de Lisboa

(Cátia Braga – tese na área do bem estar animal)

6- Novo meio de controlo de infestações pelo parasita Gasterophilus intestinalis em cavalos

(Teresa Rodrigues – Tese em colaboração com uma clínica veterinária de cavalos em regime ambulatório)

7- Avaliação de casuística de osteoartrite da articulação interfalângica proximal em cavalos

(Pedro Bastos - Tese em colaboração com uma clínica veterinária de cavalos em regime ambulatório)

Figure 14: List of the presentations for the Scientific Interaction Project