

Research article

# A pilot study about multiple congenital ocular anomalies in trait Comtois horse breed

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**Abstract:** The Silver gene is at the origin of the special coat color of the Trait Comtois Horse breed, but also predisposes carriers to Multiple Congenital Ocular Anomalies syndrome.

The objective of this pilot study was to evaluate the awareness and knowledge of Comtois owners about this topic through their response to a questionnaire. After completing it, we offered documentation for them to keep and to refer to when they feel the need.

The majority of the owners were aware about the gene, but still many are confused with its issues. Genetic testing is not systematically done when ocular problems are noticed, but willingness to pay more attention to genotypes is present in the common spirit. By developing a proper reproduction management and a proper genotyping of the Comtois horse breed population, conservation and enhancement of the breed will be possible. In this regard, Silver homozygous equines should not be discriminated, as they may possess desirable traits and may be able to perform as any other horse in their domain of activity. They should instead be encouraged to be mated with their bay peers, in order to obtain heterozygous foals. Genetic testing campaigns are increasingly offered during competitions and open to all categories of horses.

This problematic should not divide breeders, but rather motivate them to work altogether to be able to perpetuate the Trait Comtois horse breed, flagship of the Franche-Comté region.

**Keywords:** Trait Comtois, Silver gene, multiple congenital ocular anomalies

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## 1. Introduction

Being preyed on animals, visual perception is important for horses to escape danger. More than that, as they are today a very polyvalent breed, they also need this sense to complete different tasks they might be asked in domains such as fieldwork, sports, or hobbies.

Silver dilution is a dominant trait. This means that a horse requires only one parent to carry and pass on the gene. The silver dilution gene will only alter black pigmented horses (E/e or E/E) and alters the coat by diluting areas of black pigment. It has no effect on red pigmented horses (e/e). The effects of the silver dilution gene can vary greatly. When a uniform black horse is diluted by the silver gene, the mane and tail are lightened. The body is also lightened to a chocolate color, which is often dappled as well. A bay horse carrying the silver gene will usually have a lightened mane and tail, as well as lightened lower legs.

Genetic testing can be very important, as the silver gene is not always expressed. Although a red horse will not be diluted by the silver gene, it can be a carrier and pass the gene to its offspring. Because the gene is dominant, only one copy is needed for the horse to develop the silver colorations.



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For the Genotype and Phenotype there are multiple possibilities that can result according to the multiple genes involved in color patterns:

- Black with single silver (E/\_ a/a Z/N): black silver with iridociliary cysts
  - Black with double silver (E/\_ a/a Z/Z): black silver with MCOA
  - Bay with single silver (E/\_ A/\_ Z/N): bay silver with iridociliary cysts
  - Bay with double silver (E/\_ A/\_ Z/Z): bay silver with MCOA
  - Chestnut with single silver (e/e Z/N): chestnut with iridociliary cysts
  - Chestnut with double silver (e/e Z/Z): chestnut with MCOA

Comtois horses are the first draft horse breed in France. Its popularity never stopped to grow: they appeal to the large public through its gentle character, its conformation and its typical coat color. Its characteristic silver mane and tail shade was selected through the years and is linked with a mutant allele from the locus Silver, that is the gene PMEL17, inducing the dilution of the eumelanin pigment ((black and brown) [1]. Multiple congenital ocular anomalies (MCOA) is an inherited syndrome an inherited condition predominantly involving the anterior segment of the eye. Firstly demonstrated in Rocky Mountain Horse related breeds, such as Kentucky Mountain Saddle horses, Mountain Pleasure horses and Morgan horses [2], but also in unrelated breeds such as miniature horses [3], ponies [4] and Icelandic horses [5] it is also responsible for the Multiple Congenital Ocular Anomalies syndrome (MCOA), consisting in lesions localized in the anterior segment of the eye mainly (iridociliary and/or peripheral retinal cysts, cornea globosa, cataract and iris hypoplasia [2, 6-9].

The Comtois horse does not escape this rule and is thus also predisposed to these anomalies. Many foals are born blind, and mature horses are frequently seen with cornea globosa or cataract [10]. Also, in France the subject of ocular anomalies associated with the Comtois breed is evaluated through scientific surveillance and research [11-13].

However, it is possible to reduce the incidence of the disease through genetic testing and good reproduction management programs. In order to tend to the obtaining of an always healthier and sustainable breed, it comes to the responsibility of breeders to make the right choices. In this way, the goal of our study was to elaborate a questionnaire in order to point out the actual knowledge about this topic among Comtois owners.

## 2. Materials and Methods

### 2.1. Recruitment, interviewing of horse owners and questionnaire design

This pilot study was conducted through the collection of answers to a Google Form, counting 21 questions. The questionnaire was drawn up and intended for Comtois horse breeders and owners from France. Prior to its publication, a meeting with the President of the National Association of Trait Comtois horse breed was held on the 17th of June 2021 in Besançon, in order to present the project and the goals.

The questionnaire was launched in the first of August 2021 on internet and social media. A QR code leading to our questions when scanning it was also created. The responses collected were anonymous in this situation. On the other hand, breeders and owners were also personally invited to complete this survey which was presented at events such as local, regional and national Comtois breed competitions. The Google Form was closed on the first of March 2022, meaning that the study was conducted over a seven-month period.

Our main goal was to raise the awareness about the Silver gene and its implication in sight problems that might be encountered in Comtois horse. This questionnaire allowed us to realize about the popularity of this subject within the owners population. We also wanted to explain that breeding management is essential in order to avoid or decrease the obtaining of horses with ocular anomalies, since genetic stands for an important part in this field. Finally, we wished to provide an overview and an update about the topic to the members of the Comtois horse association who are implied in the improvement of the breed.

### 2.2. Questionnaire: documentation

At the end of the questionnaire, we joined two documents that explain the basics of genetics as well as a cross board for the owners to understand better what they can expect from mating when they know the genotype from both partners. In this way, it shows them that avoiding obtaining homozygous foals is possible and not that complex when we know the genotype of both parents.



**Table 1.** Overview of the number of males and females owned in the same stud farm.

Number of females owned	Number of males owned	Number of owners	Corresponding percentage
1 to 5	1 to 5	48	19.6 %
6 to 10	1 to 5	15	6.1 %
11 to 15	1 to 5	11	4.5 %
16 to 20	1 to 5	4	1.6 %
> 20	1 to 5	3	1.2 %
11 to 15	6 to 10	1	0.4 %
16 to 20	6 to 10	1	0.4 %
> 20	6 to 10	1	0.4 %
16 to 20	11 to 15	2	0.8 %
> 20	11 to 15	1	0.4 %
6 to 10	16 to 20	1	0.4 %
> 20	> 20	3	1.2%
		Total=91	Total=37%

- The third question was about the number and sex of the horses owned. According to the results, 54% owned females only, the general tendency was owning between 1 and 5; 9% owned males only, all of them owning between 1 and 5.

37% owned males and females, the general tendency was owning between 1 and 5 horses of each sex (Tab.1).

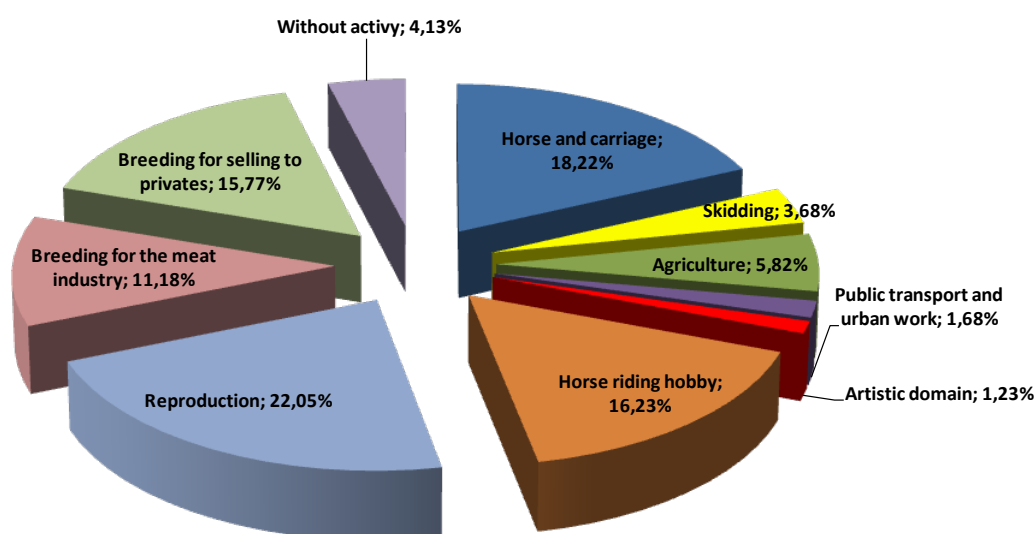
One person answered by owning no male and no female, this is the reason why the sum of the number of persons owning horses is equal to 244 and not 245. The rest of the data linked with this case were discarded because it was irrelevant for the study. Even if this person has owned Comtois horses in her career, we are rather interested in people owning some in order to have a true overview about what we can observe currently. Thus, we will continue this study by treating the 244 responses left.

The majority is owning female Comtois. This might be due to the possibility of obtaining a foal each year and thus an economic income, but also because of the ease in managing mares rather than stallions. For people owning both sexes, we can think that they answered the questionnaire in the period they were having a male foal under the mother, for example, as we did not ask about the ages of the animals. Indeed, owning intact males together with females require more installations and management, but this is not impossible. Moreover, we did not ask if the males were castrated or not, in this way owning both sexes is easy. Today, genetic testing is compulsorily done on reproductive males only. The fact that females are very well represented is important, as they are giving birth each year for most of them. They are responsible for a half of the genetic heritage of the foal to be born. Thus, they need to be taken as much in consideration as the chosen stallion when it comes to genotyping. These remarks will be discussed in more detail later.

- The fourth question was about the enrollment into the Comtois horse breeding program. The objective of the breeding program is "to allow the Comtois horse breed to improve the quality of all its representatives while promoting the marketing and development of markets and thus to provide farmers with a good level of remuneration for their products". 57.8% are enrolled in the breeding program and 42.2% are not. More than half of the sample is composed of owners breeding and raising Comtois horses to add an economic value to their activities. The rest are often privates with no economic interests.

- The fifth question concerned the domains in which the horses were performing. Ten different proposals were presented, and several boxes could be ticked in this situation. The results are presented in the following histogram (Fig. 3). Most of the horses are used in reproduction (59%), followed by horse and carriage (48.8%), which shows that this breed kept its basic usefulness until today. We can link it to the previous question, in which we showed that 57.8% of breeders were taking part in the breeding program. These results are consistent. Horse riding takes also a huge part in the activities of the breed (43.4%), showing

that it has evolved from being the farmer helper to a companion animal, used for leisure and hobbies. Through this question, we showed that this draft horse breed is very polyvalent: its uses extend from a horse used in agriculture and reproduction, to a horse appreciated by the large public.



**Figure 2.** Activity domains of the Comtois horse sample.

The second part of the questionnaire deals with the Silver gene. In this section, we are interested in the knowledge of breeders and owners about this topic.

- The sixth question concerns the knowledge of the existence of the Silver gene. The majority has ever heard about it, which represents a proportion of 73%, which is an encouraging data.

A huge proportion of our sampled owners have heard about the Silver gene. However, when we asked if they were familiar with its implication in the Comtois horse breed, 43.9% were not. In this way, it shows that this subject might be in the mind of many concerned parties but without a real understanding of the issue. From what our discussions with some breeders have shown, this is surely due to the fact that genetics is often perceived as a very specific and complex field, sometimes discouraging them to understand and learn more about this gene.

If we look closely at the portion of new breeders and owners of Comtois horse, meaning less than 5 years of experience, we calculated that 30.2% of them have never heard about this gene and do not know about its implication in the Comtois breed (Tab.2). 44.44% have heard about it and know about its implications, and 25.40% have heard about it but do not really know about its effects. It means that in 5 years of owning this type of horse, at least 69.8% are conscious of the existence of the Silver gene, which is a great progress and shows that prevention and communication about it exists. In contrast, in the category of people owning a Comtois horse for more than 15 years, 25.97% are still not aware about this problematic. We could have expected a smaller percentage in this group because of the experience they are supposed to acquire.

**Table 2.** Representative table of the experience of owners and their knowledge about the Silver gene.

Owners experience categories and knowledge about Silver gene	Number	Percentage from the considered category	Percentage from the total owners	
<b>Owners with less than 5 years of experience</b>	<b>63</b>	<b>%</b>	<b>244</b>	<b>%</b>
Heard about the Silver gene and know about its implications	28	44,44	28	11,48
Heard about the Silver gene but do not know about its implications	16	25,40	16	6,56
Never heard about the Silver gene and do not know about its implications	19	30,16	19	7,79
<b>Owners with 5 to 15 years of experience</b>	<b>77</b>	<b>%</b>		
Heard about the Silver gene and know about its implications	40	51,95	40	16,39
Heard about the Silver gene but do not know about its implications	17	22,08	17	6,97
Never heard about the Silver gene and do not know about its implications	20	25,97	20	8,20
<b>Owners with more than 15 years of experience</b>	<b>104</b>	<b>%</b>		
Heard about the Silver gene and know about its implications	67	64,42	67	27,46
Heard about the Silver gene but do not know about its implications	10	9,62	10	4,10
Never heard about the Silver gene and do not know about its implications	27	25,96	27	11,07
<b>Total</b>	<b>244</b>			

- After these first two questions of the second part of the questionnaire, we informed our readers that the Silver gene is systematically present in Comtois horses with a clear mane and tail. We added that this gene was responsible for the dilution of the coat color and was predisposing carriers to ocular anomalies. We concluded by specifying that these anomalies were more or less important according to the horse's genotype, meaning homozygous or heterozygous for the Silver gene.

- In the eighth question, we asked them if they were aware that a genetic test existed that allows them to identify if their horse is a carrier. At the same time, the way the test is performed was also exposed. The results were the following, 37.3% did not know about the existence of the genetic test; 33.6% know about the genetic test and that it can be done from blood or horsehair sampling; 29.1% know that a genetic test is possible, but they do not know how it is performed, or more precisely from what sample it is possible to do it. These data demonstrate that 62.7% of people know that genetic testing is available. As for the Silver gene, the parallel should be made directly with genetic testing when raising the awareness of Comtois owners. This way, knowledge about these tests would spread and be easily anchored in the mind of people.

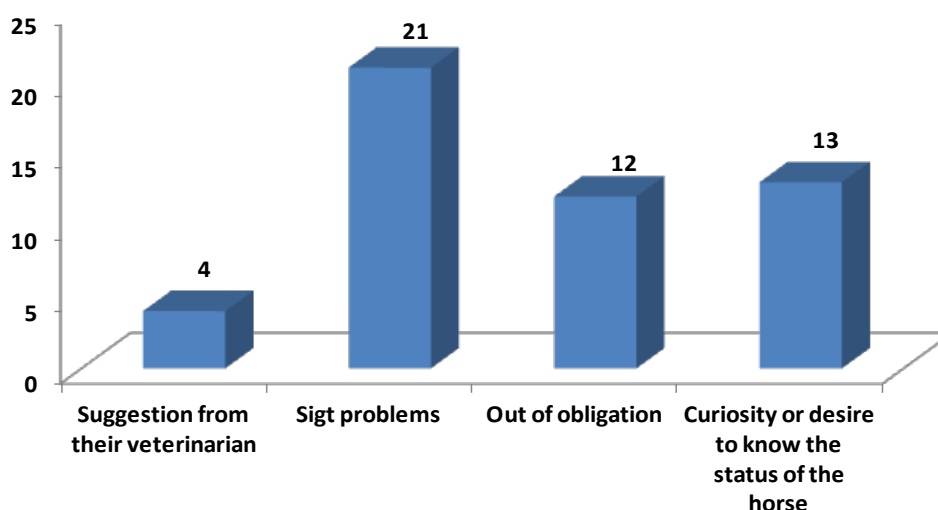
Choosing between hair or blood samples depends on the preference of the person realizing the sampling. When veterinarians are performing it during the national competition on males, blood sampling is the method realized. Also, when harvesting horsehair, caution should be made to get the hair bulb, because it is the only part that contains the genetic information. This last method of sampling is accessible to the large public, as it does not require any particular skill. A form to be completed previously is joined to the hair and send to the laboratory.

The owners were informed them that the average price of a test is around 40€ (at the time the questionnaire was developed). The ANCTC covers these costs for the males when collecting the samples on the national competition of the breed taking place in Maïche in September. For the majority, 68.9%, the answer was positive, for 14.3% it was not, and the 16.8% left were without opinion. The results are promising

because it means that owners would maybe be more ready to perform genetic testing on their horses as they consider it as affordable. Also, group prices are often proposed by laboratories when it comes to many horses to be tested, such as during events. This should further encourage in genotyping.

- In the next question, we asked owners if they had ever used these tests on their animals. We found that 84.8% never did; 7.4% did on some individuals independently of their gender; 4.9% did on stallions only and 2.9% did systematically in mares and stallions. Only 37 persons realized them, meaning 15.2%. We can observe that even if genetic testing is known by 62.7% of people, the proportion of owners that are actually carrying it out is minimal. These results show that more actions should be taken to encourage genetic testing in all animals, independently of their gender. Indeed, there is a ditch between what is known by Comtois owners and their actual actions.

Then, we detailed that in order to be approved to reproduce within the French studbook of the Trait Comtois horse, the stallion candidates must have their genotype determined before the allocation of the first breeding [14]. The results are communicated to the breeder and do not constitute in any case the removal of a stallion from reproduction, whatever its genotype. It comes down to the will and good sense of the breeder to make the proper crosses.



**Figure 3.** Distribution of owners according to the reasons they wanted to perform genetic testing.

To continue, we wanted to find out for what reasons those 37 persons were realizing genetic testing on their horses. Several boxes could be ticked, and other motivations could be added in this inquiry (Fig.3).

- The last question of this second part was about the importance owners attached to the usefulness of their horse's vision in their domain of activity. We found that 72.1% find it very important; 23% find it important; 1.2% find it of a minor importance; 3.7% are without opinion.

From these last two categories, we checked the domain of activity of the implicated animals in order to understand why these owners do not perceive the sight of their horse as essential in their activities. For the majority, they are used for reproduction only, or they do not have any particular activity. We can think that these persons do not focus on the sight of their horse because it is not primordial in their tasks, as long as they are healthy and can produce a foal each year. However, it is from healthy mares and stallions that they could obtain foals with the best genotype as possible, meaning heterozygous for the flaxen chestnut horses, or non carriers homozygous for the bay ones. For the few other owners remaining, the horses are used for horse and carriage or horse riding. Here, the utility of vision should yet be of first importance.

Sight is considered as an important to very important criterion for the majority of owners. They need to have healthy horses with a sharp sense to better help them in their daily duties or hobbies.

#### *Questionnaire : ocular anomalies*

The third part of the questionnaire deals with the ocular anomalies the owners might have encountered in their Comtois horses.

Our fourteenth and fifteenth questions were about the link between the coat color of the horses, namely flaxen chestnut or bay, and the observation of some ocular anomalies they could have such as blindness, big eyes, bad vision from afar, difficulties to locate in dark or heavily lit areas, or others. From our

analysis, we had 11 persons owning exclusively bay horses. From this sample, none declared having noticed ocular problem. We had 110 persons owning exclusively flaxen chestnut horses. From this sample, 45 declared having noticed ocular problems, meaning 40.9%; 120 persons owning flaxen chestnut horses and bay horses, out of this sample 39 never observed ocular problems for both coat colors (32.5%); 54 observed ocular problems in flaxen chestnut only (45%); 2 observed ocular problems in bay only (1.7%); 7 observed ocular problems for both coat colors (5.8%) and the 18 persons left never observed ocular problems or do not know (15%).

Three persons answered by having no bay and no flaxen chestnut horse. We excluded them for the analysis of the data coming out from this question, because it is considered as irrelevant. Indeed, some owners of horses with a particular coat color such as Silver black may have not understood that they should enter the category of flaxen chestnut in this question, as it is the Silver genetic profile we are interested in, generalized as the color of the mane and tail.

Independently of the coat color, 113 persons have observed these kinds of ocular problems in their horses, which represents 46.3%.

As a general fact, bay horses are less represented in the Comtois breed. As they are not carriers of the Silver gene, ocular anomalies reported by owners can not be linked with it. Moreover, they are a minority in this study. On the other hand, the incidence of ocular problems in flaxen chestnut is quite high : 110 persons out of 244 have ever noticed it, which corresponds to 45% of the sample.

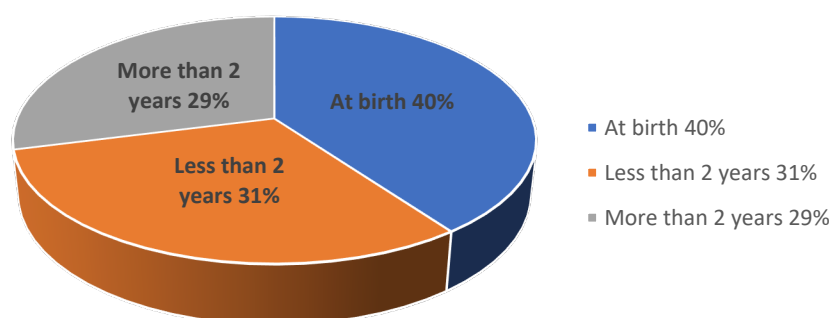
We can conclude that most of the owners have ever noticed ocular problems or at least sight deficiency in their horses. This is particularly true for flaxen chestnut Comtois.

In the following question, we asked from these 113 people who noticed ocular problems in their horses if they submitted them to a genetic test. Only 9 of them did, which represents 8% of this sample.

Realizing a genetic test will not help in resolving the vision troubles for the horses that may already have it, but it permits to make a link with their genotype and thus to be able to better apprehend future mating with the aim of decreasing the obtaining of homozygous foals. We will explain it later through the use of a combination square.

We could have added a question concerning the result of the genetic tests for owners who performed it on their horses, but anyway, answers coming from only 8% of the whole sample group would not have been enough representative.

The age at which ocular problems were noticed was asked in the next question. We chose three categories, namely at birth, before two years old or after two years old. We picked the age of two as a reference because it is the time at which males are compulsorily tested during the national competition. Thus, we wanted to know if vision problems were observed by owners before or after this age, trying not to be influenced by the results they could have got from genotyping. It was observed: at birth in 40% of the cases; in horses less than two years old in 31% of the cases; in horses more than two years old in 29% of the cases.



**Figure 4.** Age at which ocular problems were noticed by Comtois horse owners.

The MCOA syndrome is supposed to be nonprogressive, although it was described earlier that the number of cysts might increase with age but their implication in the impairment of vision is not certified as they are translucent [10].

In most of the cases, sight deficiency or ocular anomalies are noticed early after birth, with sometimes foals reported as being totally blind. The observation of vision problems in horses of more than two years

old might be because the horse was bought at this age or because the horse started to be more handled, enabling the owners to make these observations.

- The last question of this part was about the confirmation of these ocular problems by a veterinarian. For 60% of the owners, it was not confirmed by a veterinarian. From the 40% who had their veterinarian checking the eyes and confirm the ocular deficiency, uveitis was mentioned as a diagnosis from 7% of them. Uveitis represents the inflammation of the uvea, meaning the iris, the ciliary body and the choroid. This disease is not linked with the Silver gene, but can lead to vision loss on a short or a long term if it is chronic. The possible etiologies for uveitis are multiple. We consider it not relevant for our study.

#### *Questionnaire : reproduction*

In this last part of the questionnaire, we are approaching the subject of reproduction in Comtois horse, always with the Silver gene consideration.

In the eighteenth question, we asked about the importance breeders and owners were attaching to genotypes in their choices for mating. The vast majority recognize it as being important to very important. From people without opinion, 64% are not using their horse for reproduction. Therefore, this information can in part explain this outcome.

We can criticize these results. Indeed, the majority says that genetics is important in their choice for reproduction, but only a small part of breeders practice genotyping of their Comtois horses, when this is not imposed, as seen in the fifteenth question. One may wonder if there has been confusion with the phenotype of horses used in mating, many choosing stallions according to their personal attractiveness for such or such coat color.

Then, we reminded the fact that bay horses are not carriers of the Silver gene and that consequently, they are not predisposed to the development of ocular anomalies linked to it. This being known, we asked them if they would be ready to choose them more often in their choices for mating.

The survey shows that, 68.4% are ready to do it, 6.6% are not and 25% are without opinion. From our experience and our discussions on the field, because bay Comtois horses are less popular and sometimes less appreciated by some people than flaxen chestnut, it may be a factor explaining the reason why they would not favor them in reproduction.

However, the coat color resulting in the foal from crossing a bay horse to a flaxen chestnut horse will depend on the genotype of the latter. Indeed, for a homozygous, the color obtained will always be flaxen chestnut, as it will inevitably transmit an allele bearing the Silver gene. For a heterozygous, there is a 50% chance to obtain either a bay or a flaxen chestnut foal.

If we analyze the results, from the 11.1% who considered the genotype profiles not that important in their choice for mating, 63% would finally avoid crossing two homozygous horses. It means that this subject may have raised their attention, and they would be ready to consider the best options when it comes to reproduction. In contrast, always considering this pool, 15% would still not avoid crossing homozygous horses and 22% are without opinion.

The overall outcome is satisfying, with almost 70% of people who would pay attention not to mate two homozygous together. We could have added a question for the rest of the owners by asking them why they would not avoid it. We assume that sometimes the phenotype of the horses is more important to the eyes of some persons than the actual genotype of the horses.

For the last question of this study, we were interested in learning how many people were thinking that in addition to knowing the genotype of the stallion, the genotyping of breeding mares would be useful.

Most (77.5%) find it helpful to test mares for the Silver gene as well. This is encouraging, because it shows that breeders and owners are conscious of the problematic and are ready to push further the analyses. In this trend, some local Comtois breed sections are proposing to their members a genetic test on fillies and mares that can be carried out during the cantonal competition. For some, the costs are covered by the section in order to encourage the breeders to participate. This is a good opportunity for them because it would permit a better handling of reproduction by knowing the genotype from both horses and thus make rational choices in order to obtain healthy foals.

#### 4. Conclusions

This section is mandatory but can be added to the manuscript if the discussion is unusually long or complex.

Multiple Congenital Ocular Anomalies syndrome in Comtois horse is caused by a mutation on the Silver locus that codes for the typical diluted coat and hair color of this breed. It predisposes to different ocular anomalies, the severity of which depends on the Silver genotypic profile. The cystic profile corresponds to the development of iridocilliary cysts in heterozygous horses, thought to be less severe than the MCOA profile found in homozygous, in which is added cornea globosa, persistent myosis, iris hypoplasia, cataract, retinal dysplasia and detachment.

Our study goal was to report on the actual knowledge of this problematic in Comtois owners through the use of a questionnaire. We predicted to have some owners with an excellent level of knowledge on this topic, but at the same time we expected that for some of them the understanding of the issues might be difficult, and implementation of the recommendations not always followed.

The existence of the Silver gene is widely known, but the answers were more reserved when we asked if they were familiar with its implication. A bit more than half of the owners know about genetic testing and consider it affordable. However, a minor part actually perform it on their animal and generally out of obligation for stallions. In some cases, ocular problems or desire to know the status of the horse were reasons to realize testing. Since sight was considered as a very important criterion by almost all owners, genotyping could have been expected to be more common. Moreover, about half of them has ever noticed sight deficiencies in Silver bay Comtois. Also, they mentioned that they observed them at birth mainly, and in horses less than two years old. Genetic testing realized on some competition in 2020 and 2021 on young horses showed that half of them are homozygous. This result supports the two previous observations.

Genotype of the horses used in reproduction was considered important by the majority of owners. Yet, this result can be criticized as genetic testing is not enough carried out when it is not compulsory.

After reminding breeders that bay Comtois are not carriers of the Silver gene, two third were ready to use them more often in mating. The same result was obtained when it came to avoid crossing two known homozygous horses together. Finally, more than three quarters of Comtois owners think that it would be useful to genotype mares in addition to stallions.

Sight problems are clearly present in the Trait Comtois breed, and these are noticed by owners. The outcome of this study is encouraging concerning Silver gene management in reproduction. Indeed, in the last part of our questionnaire, after reminding some information, the majority answered with the will to care about the transmission of it.

In the last part presented thereafter, we would like to propose some solutions and some plans to be implemented in the future in order to try to alleviate the occurrence of MCOA syndrome in Trait Comtois horse breed.

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