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Facultad de Ciencias Veterinarias

ORIGINAL ARTICLES

SHORT COMMUNICATIONS

CASE REPORT

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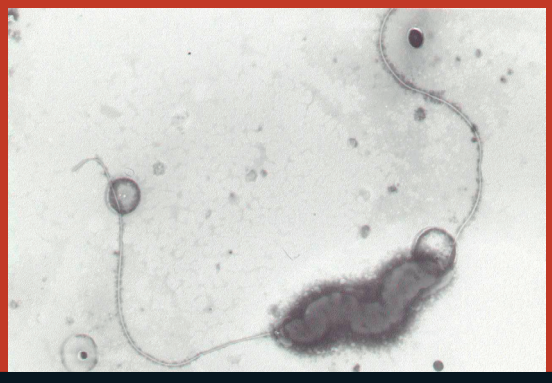

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ORIGINAL ARTICLE

Citizens' attitudes and perceptions towards genetically modified food in Chile: Special emphasis in CRISPR technology

Tamara Tadich[a], Sebastián Escobar-Aguirre[b]*

ABSTRACT. To date, there has been an increase in genome modification biotechnologies that improve production and food security but the process has not been accompanied by the delivery of information about them intended for citizens. This is essential considering that to achieve better health, food security and sustainability these biotechnologies need to be incorporated into production systems. This study aimed to explore perceptions and attitudes of Chilean citizens towards the use of genome modifications with an emphasis on transgenes and genome editing (CRISPR). An electronic questionnaire was applied, and afterwards the results were analysed through descriptive statistics, GLM, Spearman's correlation and Wilcoxon Signed Rank test. A total of 702 questionnaires were analysed. High awareness of concepts such as transgenic and cloning was reported with CRISPR being the least known term. Most respondents perceived negative effects on health regarding the consumption of genetically modified products, with women having a significantly more negative attitude. Still, a high willingness to use CRISPR for improving animal and human health was reported. When comparing vegetable and animal products that underwent CRISPR or transgenes, the willingness to consume these products was higher for vegetables. The results show that changes in perception can be achieved after providing the definition of CRISPR and transgenic, therefore, consumer education seems to be essential. Science communication focused on making information about genome modification biotechnologies available to citizens could promote more positive attitudes and perceptions and facilitate their future implementation in the country.

Key words: attitudes, CRISPR, genetically modified food, perceptions.

INTRODUCTION

Genetic modification (GM) allows novel traits to be introduced in the agricultural sector in organisms such as fish, livestock, poultry and crops to improve their productivity. Historically, the introduction of the first genetically modified food into the US market began in 1996 with GM maize (Cui and Shoemaker 2018). Since then, new genetic approaches are being continuously developed and implemented in the food chain, promising more efficient production and better quality for consumers (Dong and Ronald 2019). These technologies (GM) allow enhanced nutritional value (i.e. biofortified crop), economic, and agronomic benefits (i.e. lower use of pesticides) among others (Dong and Ronald 2019). However, the estimated increase in global population -10 billion people by 2050- has provoked an enormous pressure on food supply, which is amplified by the limitation of arable land, global warming effects and limited water resources (Cui and Shoemaker 2018). In a recent review by Menchaca (2021), emphasis is given to the challenge of increasing productivity while conserving the environment and biodiversity. In this context, new alternatives and disruptive technologies need to be implemented to ensure food security and sustainability of food production systems. Among these, the advent of clustered regularly interspaced short palindromic repeats (CRISPR) has proved to be a powerful and precise tool for editing specific regions of plant and animal genomes (Bartkowski *et al* 2018, McFarlane *et al* 2019). Contrary to transgenics, this new mechanism can create precise incisions, mutations and substitutions in the genome of plant and animal cells with no new foreign DNA being added.

Nowadays, the CRISPR/Cas system has been successfully applied for genome editing in soybean (Jacobs *et al* 2015, Li *et al* 2015), maize (Svitashev *et al* 2016), tobacco, lettuce, rice (Woo *et al* 2015), and animals, including chicken (Véron *et al* 2015), rabbits (Kawano and Honda 2017), pigs (Hai *et al* 2014, Whitworth *et al* 2014), goats (Ni 2014), sheep (Crispo *et al* 2015), cattle (Gao *et al* 2017) and later to fish (Li *et al* 2021). Therefore, the development of GM foods (livestock species and crops) using CRISPR is one of the most realistic solutions considering the current global scenario. However, the debate for creating new genetically modified organisms (GMOs) is a permanent cause of concern among people and potential consumers (Schnettler *et al* 2008, 2012, 2016, Zhang *et al* 2016, Popek and Halagarda 2017, Bruetschy 2019).

In general terms, the debates over GM foods are focused on public awareness about the potential adverse effects on human health and the environment (Cui and Shoemaker 2018, Hanssen *et al* 2018). This uncertainty can be explained by deficient and ambiguous science communication strategy to the public; ethical and moral perceptions, and trust in governments and scientists (Shew *et al* 2018), all of which ends having an impact on perceptions and attitudes. Perceptions can be understood as the way a person interprets stimuli into something meaningful, although this interpretation can be substantially different from reality,

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while attitudes are a mental state of readiness towards something, it depends on perception and will influence decision making and guide behaviours (Pickens 2005). Previous work has shown public opposition to GMOs, probably the most studied case was the "GM Nation?" public engagement exercise that has been described as a "mess" (Tait 2001). This opposition has also been described in China where negative perceptions have been reported in 46.7% of surveyed people (Cui and Shoemaker 2018) and among the Dutch population approval is only around 30% (Hanssen *et al* 2018). However, and contrary to the negative perception of GMO food, the medical applications of GM were endorsed by 70% in the same report in Germany (Hanssen *et al* 2018). The perceived benefits and risks of GM are the main reason for certain attitudes towards genetic manipulation. For example, animal health and welfare can affect positively attitudes toward genome modifications in dairy cows in Canada (Ritter *et al* 2019), a similar conclusion was observed in a Japanese survey but related to genetic disease in humans and the use of gene therapy (Uchiyama *et al* 2018). In Chile, poor knowledge of the meaning of transgenic and a negative perception of animal production technologies has been reported before (Schnettler *et al* 2012, 2016), with a lower acceptance of food including beef or milk obtained through genetic modification and cloning. Nevertheless, the perceptions of Chilean citizens about CRISPR have not been studied.

In some countries, such as Canada, CRISPR is not subject to the conventional regulations of a genetically modified organism because no foreign DNA is added. It is important to open this local discussion to establish a new frame of science-policy implementation in genome editing, especially in Chile where it has not been determined the regulatory status of gene editing in animals yet.

With this in mind, this study aimed to explore, for the first time in Chile, the perceptions and attitudes of citizens towards genetically modified products with special emphasis on transgenes and genome editing in the agriculture and animal farming industries.

MATERIAL AND METHODS

To assess attitudes and perceptions towards genome modification biotechnologies a questionnaire was constructed in Spanish using the Google Forms tool (Google ® California, USA). The questionnaire consisted of five sections and accepted only one response per user, respondents had to be over 18 years old. Section one included the informed consent, then participants were asked about their demographic information (gender, age, region of residence, diet and education level). Section two contained general closed-ended questions about knowledge of different genetic modification tools and perceptions about their use. Section three focused on transgenic products, the definition of transgenic was provided and then seven closed-ended questions about their attitudes towards some vegetable and animal transgenic products. The fourth section on genome editing was included with the same seven questions provided in section three. Finally, section five consisted of one open-ended question where participants were asked to name genetically modified products of vegetable or animal origin that they perceive are present in the Chilean market.

The sample size was determined a priori assuming 95% power at an alpha of 0.05. The data from the last national demographic survey[1] (INE 2017) was used for the total number of households in Chile; a sample size of 385 surveys was estimated. Survey participants were recruited through social media such as Facebook, Instagram, Whatsapp (Facebook Inc., Menlo Park, CA, USA) and Twitter (Twitter Inc., San Francisco, CA, USA). The questionnaire was open between April and May of 2020. After closing the form, data was downloaded into an Excel spreadsheet and frequencies, means, standard deviations, and percentages were calculated. To evaluate the effect of demographic variables on perceptions a GLM was used, correlations were assessed using Spearman's correlation and Wilcoxon Signed Rank test was used to evaluate differences in perception of transgenes and genome edited products before and after providing the definitions. A *P*-value lower or equal to 0.05 was used to establish the significance of the results, the statistical software Minitab® 19 (PA, USA) was used. For the open-ended question, a frequency analysis was used.

RESULTS

A total of 709 respondents agreed to answer the questionnaire, from these 702 were included in the analysis and 7 were eliminated due to incomplete questionnaires or declaring ages below 18 years. The demographic characteristics are described in table 1. A similar percentage of responses was retrieved from females and males, with most participants being in the age range between 18 and 40 years of age. Most respondents had completed a technical or professional career, had an omnivore diet and were from the Province of Santiago in the Metropolitan region (table 1). Figure 1 shows that the most known tool for genetic modification was transgenes (97.4%), while the least known one was CRISPR (33.8%).

PERCEPTIONS PRIOR TO DELIVERY OF BIOTECHNOLOGIES DEFINITIONS

When asked about the possible negative effects of genetic modification tools on other animals or vegetables 64.8% perceived they do have a negative effect, 9% that they do not, 23.8% perceived that maybe and 2.4% do not know.

[1] INE, Instituto Nacional de Estadísticas. 2017. https://www.ine.cl/ine-ciudadano/definiciones-estadisticas/censo#:~:text=Resultados%20definitivos%20CENSO%202017,51%2C1%25)%2C%20mujeres.

Table 1. Number and percentage of respondents according to socio-demographic characteristics (n=702).

	Number	Percentage
Gender		
Female	344	49.0
Male	346	49.3
Non-binary	12	1.7
Age		
18-40	376	53.6
41 to 60	213	30.3
> 60	113	16.1
Education		
Did not complete high school?	0	0
Completed high school?	52	7.4
Completed a technical or professional degree?	413	58.8
Completed a postgraduate degree?	237	33.8
Diet		
Omnivore	599	85.3
Vegetarian	82	11.7
Vegan	21	3.0
Macrozone of residence*		
North	18	2.6
Center-North	70	10.0
Metropolitan	451	64.2
Center-South	86	12.3
Austral-South	77	11.0

*Chilean macrozones are classified in: North which includes the Regions of Arica y Parinacota, Tarapacá, Antofagasta and Atacama; Center-North including the Regions of Coquimbo and Valparaíso; Metropolitan formed by the Metropolitan Region; Center-South macrozone formed by the Regions of Libertador Bernardo O'Higgins, Maule, Bio-Bio and Ñuble; and the Austral-South macrozone formed by the regions of La Araucanía, Los Ríos, Los Lagos, de Aysén and Magallanes.

With regard to the products' labelling systems present in Chile that identify GM products in the market, most respondents declared that they are not adequate neither clear (84.6%), only 2.6% believe they are adequate and clear, and 12.8% did not know.

Figure 2 shows the responses before providing the definitions of transgenics and CRISPR. Over 60% of respondents declare that GM products can or may have adverse effects on health, and 65.2% declared there are ethical problems associated with the use of CRISPR in animals. When asked if they would agree to using CRISPR for improving animal health 43.6% said yes and 23.8% maybe. Agreement in using CRISPR for improving human health was lower (39.7% yes; 22.6% maybe).

A significant effect of gender on agreeing on the of use genome editing (CRISPR) in humans (P=0.033) and in animals (P=0.001) and on the perception of a negative effect of GM products on human health (P=0.002) was found, with women having a lower agreement and more negative perception (table 2). No effects were found for type of diet and education level.

Significant correlations were found between age and perceiving there are ethical problems with the use of CRISPR (P=0.005, r=0.11) and between age and the use of transgenic products (P=0.04, r=0.08). Also, a significant correlation was found between those respondents perceiving ethical problems with the use of genome editing and those that perceived an ethical problem with the use of transgenic products (P<0.001, r=0.52).

PERCEPTIONS AFTER DELIVERY OF BIOTECHNOLOGIES DEFINITIONS

After providing respondents with the definition of genome editing (CRISPR) and transgenic, they were asked how close these definitions were to their previous

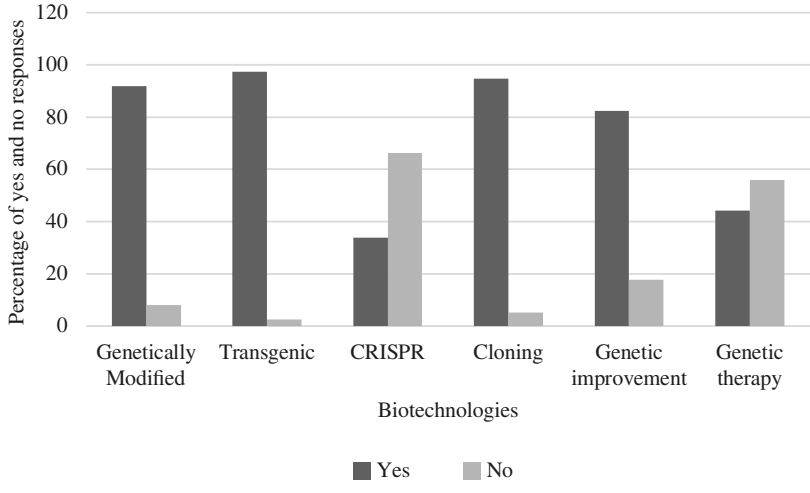

Figure 1. Distribution of respondents (n=702) according to if they had (yes) or not (no) heard previously the concepts Genetically modified, Transgenic, CRISPR, Cloning, Genetic improvement and Genetic therapy.

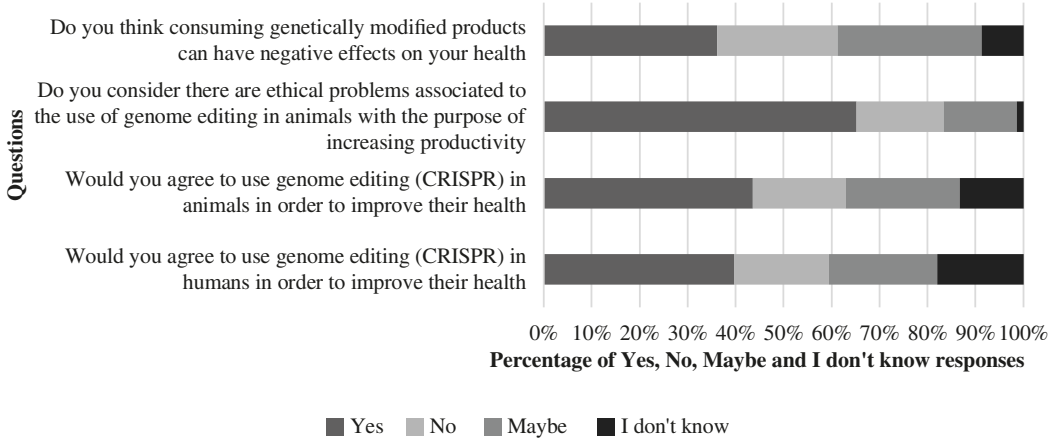

Figure 2. Distribution of respondents in percentages according to their responses (yes, no, maybe and do not know) to each question related to the use of genome editing, before providing them with the definition of genome editing.

Table 2. Results of the GLM (*F* value and P-value) for determination of the effect of gender, diet and education on responses to each question.

Questions	Gender		Diet		Education	
	F	P	F	P	F	P
1. Would you agree to use genome editing (CRISPR) in humans to improve their health.	3.43	0.033	0.24	0.787	0.14	0.872
2. Would you agree to use genome editing (CRISPR) in animals to improve their health.	0.762	0.001	0.27	0.762	0.18	0.839
3. Do you think consuming genetically modified products can have negative effects on health.	6.29	0.002	1.34	0.263	0.99	0.373

own concepts of genome editing and transgenic. At five points Likert scale was used, where 1 corresponded to not similar at all and 5 was completely the same. Regarding CRISPR, 25.6% said it was the same definition (5) they had before to the questionnaire and 12.3% said it was completely different to what they thought firstly (1); while 11.1% gave a score of 2 points, 27.2% scored it with 3 points and 23.8% scored the similarity with 4 points. For the definition of transgenic 45.44% said it was the same definition (5) and 1.71% said it was completely different; while 1.99% scored it with 2 points, 14.81% with 3 points and 36.04% scored the similarity with 4 points.

When asked about the willingness to eat genome edited food products (figure 3), more people were willing to eat vegetables developed through this biotechnology (43.2% corn and 39.2% soybean) than animal products (25.4% salmon and 24.8% beef). The same tendency was observed for transgenic food products, with people being more willing to accept this biotechnology in the case of vegetables (50.3% corn and 46.3% soybean) than for animal products (26.8% salmon and 24.9% beef).

To understand their attitudes, respondents were asked to put themselves in the following situation; if genome editing allows providing salmons with resistance to disease or to certain pathogens, would you agree to use it? Overall, 39% agreed, 32.9% did not agree, 5.7% said maybe and 22.4% did not know. Afterwards, those that responded "yes" or "maybe" (n=431) were asked if they would be willing to consume this kind of salmon; 53.4% said they would be willing to consume it, 16.9% said they would not and 29.7% said they did not know if they would consume genome edited salmon.

After providing the definition of genome editing and transgenic, there were significant changes ($P<0.05$) for both concepts when asking the question "do you think there are ethical problems associated with the use of genome editing (CRISPR)/transgenes in animals in order to increase productivity?". In the case of CRISPR, respondents that thought there was no ethical problem increased from 15.2% to 18.2%, and those that said they did not know decreased from 29.8% to 1.4% ($P=0.001$). For transgenic food products, there was an increase (44% to 52%) in respondents that perceived an ethical problem in the production of transgenic organisms, a decrease in those that did not perceive an ethical problem (23.4% to 17.52%) and those that thought that maybe there is an ethical problem (27.6% to 1.99%), while those that did not know increased from 5% to 28.34% ($P=0.02$).

When asked about how beneficial they considered the use of CRISPR for the production of vegetable or animal-based food products, in a 5 points Likert scale, 8% said it was not beneficial at all (1) and 21.4% said it was very beneficial (5); while 9.7% scored the benefit with 2 points, 34.8% with 3 points and 26.2% with 4 points. Finally, when asked to mention GM products available in the Chilean market the most frequent vegetable products mentioned were corn, soybean and tomato. Animal origin products such as chicken, salmon and beef were mentioned in a lower frequency (table 3).

Table 3. Most frequent words used when asked to mention GM food present in Chilean markets. Only those with a frequency over 10 are included in the table.

Frequency	Word (English)
144	corn
123	soybean
123	tomato
84	fruit
80	seeds
64	greens
40	wheat
27	chicken
21	vegetables
17	cereals
17	salmon
15	canola (rapeseed)
15	meat
13	bovines

DISCUSSION

Citizens attitudes and perceptions about the use of different GM biotechnologies applied in animal and vegetable origin products was assessed through a questionnaire. The electronic application of the questionnaire was preferred since it is less time consuming and expensive than face to face interviews (Heerwegh 2009). On the other hand, online questionnaires can decrease social desirability which tends to be higher during face-to-face interviews, especially considering that issues regarding genetic modifications tend to have an ethical component.

There was a balance between responses of women and men, with most respondents being between 18 and 40 years of age and with a technical or professional degree. These ages are within the generation Z, X and millennials, and it can explain the higher number of responses from these groups since they are the ones that most use social media in Chile according to CADEM[2]. The same survey shows that Twitter, Facebook and Whatsapp are the most used apps for sharing opinions, possibility that surveys provide. Only 15% of respondents were either vegetarian or vegan, results that are in agreement with the national survey on lifestyles of Chileans conducted in 2018, where 18% of respondents were either vegetarian or vegan[3].

To assess the current knowledge on terminologies associated with genetic biotechnologies, participants were asked if they had or not heard six different terms. The most acknowledged terms were transgenic and cloning with over 95% declaring that they had heard them. On the other hand, genome editing (CRISPR) was the least known term,

[2] CADEM. 2019. *El Chile que viene. Uso de redes sociales*. Available at: https://www.cadem.cl/encuestas/el-chile-que-viene-uso-de-las-redes-sociales/

[3] CADEM. 2018. *CADEM 2018. El Chile que viene*. Available at: https://www.cadem.cl/encuestas/el-chile-que-viene-abril-2018/

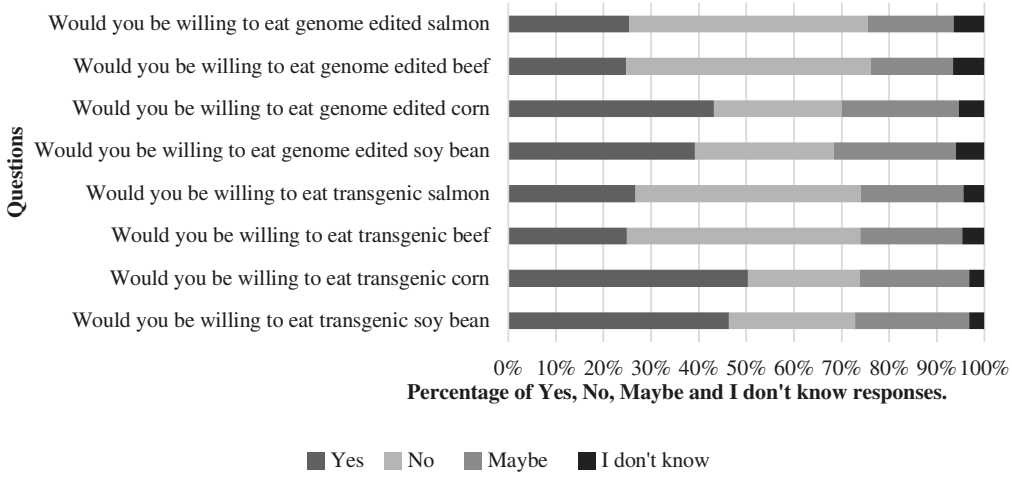

Figure 3. Responses given after reading the definition of genome editing (CRISPR) and transgenic about willingness to consume salmon, beef, corn and soybean subjected to these tools.

similar to the findings of Uchiyama (Uchiyama *et al* 2018) where only 6.6% per cent of respondents, with no genetic disease, had heard this term. It is worth noticing that fewer people had heard the term genetically modified organism (GMO) than cloning and transgenic considering that the definition of GMO is "an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination" (Bruetschy 2019) since both cloning and transgenic involve genetic modifications. This reflects the lack of information that citizens have in general about different labelling systems and their meaning. For example, Schnettler *et al* (2012) reported that 50% of consumers from the centre-south of Chile said they had received information related to GM products, but less than 20% were able to define it correctly.

All these biotechnologies raise many questions among the public, especially related to potential risks (i.e. *is it safe?*) and ethical concerns (i.e. *who benefits from this technology?*) (Bruetschy 2019), thus, information relative to them should be socialized through simple, clear and upstream communication processes (Wilsdon and Willis 2004). A study in Turkey reported that only 1% of respondents had never heard the term GMO, still they had little knowledge about this technology (Mürsel *et al* 2015). In the present study only 8% had never heard the term but being aware of the term does not necessarily imply a deep understanding of it.

When analyzing demographic characteristics and attitudes towards the use of these biotechnologies, women had a significant more negative attitude about the use of genome editing in humans and animals, and also considered that consuming GM products could have negative effects on health. Most literature on GMOs shows that women are more sceptical about the use of these products and their possible negative consequences on health (Moerbeek and Casimir 2005, Saher *et al* 2006, Heiman *et al* 2011). It also seems that women tend to think about the long-term risks of these biotechnologies while men focus on the short-term benefits (Moerbeek and Casimir 2005). With regard to diet type, contrary to our results (Saher *et al* 2006) found that meat eaters had a more positive view on GM products than meat avoiders. In the present study, no association was found between having a negative attitude towards consumption of GM products and type of diet.

Previous studies have found a relationship between higher education and greater knowledge about GMOs (Moerbeek and Casimir 2005, Saher *et al* 2006, Heiman *et al* 2011, López *et al* 2016). Popek and Halagarda (2017) also reported a lower level of knowledge regarding GMOs within citizens less educated, thus people with higher education, especially from the field of natural sciences could be more familiarised with the terms and understand better these biotechnologies. In the present study, no effect of education level was found, this could be because over 80% of respondents had completed a technical or professional degree and in some cases with postgraduate studies. Saher *et al* (2006) found that the leader predictor for attitudes towards GMOs was the field of study, with students from natural sciences having more positive attitudes. Still, the authors emphasise that multiple factors contribute and interact in GM attitudes (Saher *et al* 2006), this study did not include a question about the field of study. Nevertheless, Schnettler *et al* (2012) concluded that there is not a profile for consumers that approve or reject GM products.

Despite the low level of awareness of the term genome editing (CRISPR), 43.9% of respondents would be willing to use this technique for targeting animal health problems and 39.7% for human health. Similar results were described in Japan for its use in humans (Uchiyama *et al* 2018). Contrary to this, 65.2% of participants did consider there is an ethical problem when using this biotechnology for improving productivity. The present results are similar to previous studies where people were more supportive of using genome editing as a therapeutic tool than using it for improving certain traits (McCaughey *et al* 2016, Weisberg *et al* 2017).

After providing respondents the definition of genome editing (CRISPR) and transgenic, 25.6% and 45.44% of them said it was the same definition they had before the survey, respectively. Fewer respondents being aware of CRISPR can be explained because it is a newer biotechnology developed in 2013 (Doudna and Charpentier 2014) while transgenics were introduced in the 1980-90s (Zhang *et al* 2016). Despite the novelty of this technique, around 30% of respondents agreed that the definition of genome editing provided was the same they had.

Although this is the first Chilean report about the knowledge and perceptions about CRISPR, our results showed that Chilean respondents are more familiarised with its definition compared to Japanese participants, where 6% of respondents had heard the term (Uchiyama *et al* 2018). Conversely to previous reports in Chile, we found a higher number of respondents that knew the meaning of transgenic compared to the less than 30% reported by (Schnettler *et al* 2012, 2016). This difference might be explained by the higher education level of respondents from the present study, while in Schnettler *et al* (2012, 2016) most respondents had not completed a technical or professional degree, and half of the sample were students.

When analysing the willingness to eat GM food, the type of organism (plant or animal) is relevant for public opinion (Kronberger *et al* 2014). This has been observed with US participants in which plant-to-plant gene combination received more support than to animal-plant combination (Frewer *et al* 2004). Indeed, in a study about societal aspects of genetically modified foods, it is suggested that plant and microorganism genetic modifications are more accepted compared to those in animals (Frewer *et al* 2004). This could explain our result, where more people were willing to eat vegetables than animal products subjected to transgenic or gene editing methods. It must be highlighted that although

numerous recent studies have described the potential uses of CRISPR in animal and crop production, there is scarce information on the public acceptance of this technique. In this regard, a multi-country and massive survey found that in the USA, Canada, Belgium, France, and Australia, 56, 47, 46, 30, and 51% of respondents, respectively, were willing to consume both GM and CRISPR food (Shew *et al* 2018). Considering the mentioned report, the present results fit very well with Australia and Belgium percentage of willingness. Our result reveals that both transgenic and CRISPR technology are perceived in a similar way, even though their definitions are quite different. A similar conclusion was obtained by Shew and collaborators on their global CRISPR versus GMO public acceptance and valuation study (Shew *et al* 2018).

It is well documented that the perceived benefits and risks of GM shift the public attitudes towards genetic manipulation. For example, a majority of respondents accept genetic changes with the purpose of improving animal health and welfare, but when related to genetic disease in humans this receptivity decreases (Robillard *et al* 2014). In this study, the hypothetical situation *if genome editing allows providing salmons resistance to disease or certain pathogens, would you agree to use it?* a total of 39% of respondents agreed (yes and maybe), similar results were obtained for cattle genetically modified perception in the USA (McConnachie *et al* 2019). However, followed by the question *would you be willing to consume these salmon?* only half of this group agreed. This trade-off attitude in the Chilean respondents reflects the conflict and the thinking process to which they were faced. Even in the "positive perception toward genetic modification sub-group" we constated that a big proportion of them are not willing to eat this food. When looking into the detail of these respondents, vegan people were not represented in this sub-sampling, but among people willing to consume genome editing salmon a big proportion were men averaging 41 years of age, with college and postgraduate education levels.

It is interesting to note that the most frequent responses obtained regarding which GM product people knew were corn, soybean and tomato. These products are in line with the GM seed production in Chile where maize, canola and soybean have been the main products for export (Sánchez and León 2016); while cotton, table grapes and tomato represent less than 1% of the total area of GM seed (Sánchez and León 2016) present in the country. For this reason, it calls our attention the high frequency of tomato in this study. This misconception about GM tomato produced in Chile is assumed perhaps on the prolonged breeding technologies applied, where researchers are creating new traits and varieties of tomatoes worldwide. We must point out, that there is no GM crop production for food, human consumption or seed, for the domestic market in Chile. Therefore, the presence of fruit, seeds, greens, wheat, vegetables, cereals and canola (rapeseed) on the list represents a lack of knowledge about the regulatory framework by Chilean citizens. In this regard, Salazar *et al* (2019) concluded that the restricted commercial use to seed-export has made Chile a seed nursery to GM products. On the other hand, in this study some animal species were identified including chicken, salmon and beef. Despite the lower frequency of appearance, again the wrong idea that GM products of animal origin make it into the Chilean market is present. To date, salmon is the first genetically engineered EG animal approved for human consumption, but only in the United States and Canadian markets[4] (FDA 2015).

In conclusion, this study shows that among Chilean citizens there is a high awareness of concepts such as transgenic, cloning and genetically modified, and low awareness of the CRISPR and genetic therapy concepts. Most respondents perceived possible negative effects on health regarding the consumption of GM products, with women having a significantly more negative attitude towards them. Still, a high willingness to use CRISPR for improving animal and human health was reported. When comparing vegetable and animal products that underwent CRISPR or transgenes, the willingness to consume these products is higher for vegetables than for animal origin products. Finally, education through the provision of clear information seems to be essential. For example, in the present study perception was significantly changed after providing the definition of CRISPR and transgenes concepts.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest neither with persons or institutions.

ETHICAL STATEMENT

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REFERENCES

Bartkowski B, Theesfeld I, Pirscher F, Timaeus J. 2018. Snipping around for food: Economic, ethical and policy implications of CRISPR/Cas genome editing. *Geoforum* 96, 172-180.

Bruetschy C. 2019. The EU regulatory framework on genetically modified organisms (GMOs). *Transgenic Res* 28, 169-174.

[4] FDA. 2015. AquAdvantage Salmon Approval Letter and Appendix. Available at: https://www.fda.gov/animal-veterinary/animals-intentional-genomic-alterations/aquadvantage-salmon.

Crispo M, Mulet AP, Tesson L, Barrera N, Cuadro F, *et al.* 2015. Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes. *PLoS ONE* 10, e0136690.

Cui K, Shoemaker SP. 2018. Public perception of genetically-modified (GM) food: A nationwide Chinese consumer study. *Npj Sci Food* 2, 1-8.

Dong OX, Ronald PC. 2019. Genetic engineering for disease resistance in plants: Recent progress and future perspectives. *Plant Physiol* 180, 26-38.

Doudna JA, Charpentier E. 2014. The new frontier of genome engineering with CRISPR-Cas9. *Science.* 346, 1258096.

Frewer L, Lassen J, Kettlitz B, Scholderer J, Beekman V, *et al.* 2004. Societal aspects of genetically modified foods. *Food Chem Toxicol* 42, 1181-1193.

Gao Y, Wu H, Wang Y, Liu X, Chen L, *et al*. 2017. Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. *Genome Biol* 18, 1-15.

Hai T, Teng F, Guo R, Li W, Zhou Q. 2014. One-step generation of knockout pigs by zygote injection of CRISPR/Cas system. *Cell Res* 24, 372-375.

Hanssen L, Dijkstra AM, Sleenhoff S, Frewer LJ, Gutteling JM. 2018. Revisiting public debate on genetic modification and genetically modified organisms. Explanations for contemporary Dutch public attitudes. *J Sci Commun* 17, 1-20.

Heerwegh D. 2009. Mode differences between face-to-face and web surveys: an experimental investigation of data quality and social desirability effects. *Int J Public Opin Res* 21, 111-121.

Heiman A, Agmon O, Fleisher R, Zilberman D. 2011. Attitude and purchasing decisions regarding genetically modified foods based on gender and education. *Int J Biotechnol* 12, 50-65.

Jacobs TB, La Fayette PR, Schmitz RJ, Parrot WA. 2015. Targeted genome modifications in soybean with CRISPR/Cas9. *BMC Biotechnol* 15, 1-10.

Kawano Y, Honda A. 2017. Gene targeting in rabbits: single-step generation of knock-out rabbits by microinjection of CRISPR/Cas9 plasmids. *Methods Mol Biol* 1630, 109-120.

Kronberger N, Wagner W, Nagata M. 2014. How natural is "more natural"? the role of method, type of transfer, and familiarity for public perceptions of cisgenic and transgenic modification. *Sci Commun* 36, 106-130.

Li M, Dai S, Liu X, Xiao H, Wang D. 2021. A detailed procedure for CRISPR/Cas9-mediated gene editing in tilapia. *Hydrobiologia* 848, 3865-3881.

Li ZS, Liu ZB, Xing AQ, Moon BP, JP, Koellhoffer, *et al*. 2015. Cas9-guide RNA directed genome editing in soybean. *Plant Physiol* 169, 960-970.

López OAM, Pérez EF, Fuentes EES, Luna-Espinoza I, Cuevas FA. 2016. Perceptions and attitudes of the Mexican urban population towards genetically modified organisms. *Br Food J* 118, 2873-2892.

McCaughey T, Sanfilippo PG, Gooden GEC, Budden DM, Fan L, *et al.* 2016. A global social media survey of attitudes to human genome editing. *Cell Stem Cell* 18, 569-572.

McConnachie E, Hötzel MJ, Robbins JA, Shriver A, Weary DM, *et al*. 2019. Public attitudes towards genetically modified polled cattle. *PLoS One* 14, 1-15.

McFarlane GR, Salvesen, HA, Sternberg A, Lillico SG. 2019. On-farm livestock genome editing using cutting edge reproductive technologies. *Front Sustain Food Syst* 3, 1-7.

Menchaca, A. 2021. Sustainable food production: The contribution of genome editing in livestock. *Sustainability* 13, 6788.

Moerbeek H, Casimir G. 2005. Gender differences in consumers' acceptance of genetically modified foods. *Int J Consum Stud* 29, 308-318.

Mürsel T, Melek B, Alev Y, Nese SY. 2015. Consumer awareness, perception and attitudes towards genetically modified foods in Turkey. *Br Food J* 117, 1426-1439.

Ni W, Qiao J, Hu S, Zhao X, Regouski M, *et al*. 2014. Efficient gene knockout in goats using CRISPR/Cas9 system. *PLoS ONE* 9.

Pickens J. 2005. Attitudes and perceptions. In: Borkowski N (ed). *Organizational Behavior in Health Care*. Jones and Bartlett Publishers, Sudbury, USA. 43-75.

Popek S, Halagarda M. 2017. Genetically modified foods: Consumer awareness, opinions and attitudes in selected EU countries. *Int J Consum Stud* 41, 325-332.

Ritter C, Shriver A, McConnachie E, Robbins J, von Keyserlingk MAG, *et al*. 2019. Public attitudes toward genetic modification in dairy cattle. *PLoS One* 14, 1-15.

Robillard JM, Roskams-Edris D, Kuzeljevic B, Illes J. 2014. Prevailing public perceptions of the ethics of gene therapy. *Hum Gene Ther* 25, 740-746.

Saher M, Lindeman M, Hursti UKK. 2006. Attitudes towards genetically modified and organic foods. *Appetite* 46, 324-331.

Salazar MP, Valenzuela D, Tironi M, Gutiérrez RA. 2019. The ambivalent regulator: the construction of a regulatory style for genetically modified crops in Chile. *Tapuya Lat Am Sci Technol Soc* 2, 199-219.

Sánchez MA, León G. 2016. Status of market, regulation and research of genetically modified crops in Chile. *N Biotechnol* 33, 815-823.

Schnettler B, Sepúlveda B, Ruiz D. 2008. Acceptance of transgenic milk in La Araucania Region,Chile. *Chilean J Agric Res* 68, 380-390.

Schnettler B, Miranda H, Sepúlveda J, Denegri M. 2012. Preferências dos consumidores aos alimentos geneticamente modificados de origem animal e vegetal no Chile. *Cienc Tecnol Aliment* 32, 15-25.

Schnettler B, Velásquez C, Lobos G, Orellana L, Sepúlveda J, *et al*. 2016. Acceptance of beef obtained through genetic modification and cloning in university students and working adults in southern Chile. *Rev Fac Ciencias Agrar* 48, 141-159.

Shew AM, Nalley LL, Snell HA, Nayga RM, Dixon BL. 2018. CRISPR versus GMOs: Public acceptance and valuation. *Glob Food Sec* 19, 71-80.

Svitashev S, Schwartz C, Lenderts B, Young JK, Mark A. 2016. Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. *Nat Commun* 7, 13274.

Tait J. 2001. More faust than Frankenstein: The European debate about the precautionary principle and risk regulation for genetically modified crops. *J Risk Res* 4, 175-189.

Uchiyama M, Nagai A, Muto K. 2018. Survey on the perception of germline genome editing among the general public in Japan. *J Hum Genet* 63, 745-748.

Véron N, Qu Z, Kipen PAS, Hirst CE, Marcelle C. 2015. CRISPR mediated somatic cell genome engineering in the chicken. *Dev Biol* 407, 68-74.

Weisberg S, Badgio D, Chatterjee A. 2017. A CRISPR New world: Attitudes in the public towards innovations in human genetic modification. *Front Public Health* 5, 117.

Whitworth KM, Lee K, Benne JA, Beaton BP, Spate LD, *et al.* 2014. Use of the CRISPR/Cas9 system to produce genetically engineered pigs from *in vitro*-derived oocytes and embryos. *Biol Reprod* 91, 78-79.

Wilsdon J, Willis R. 2004. *See-through Science. Why public engagement needs to move upstream.* HenDI Systems, London, UK.

Woo JW, Kim J, Kwon SI, Corvalán C, Cho SW, *et al*. 2015. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nat Biotechnol* 33, 1162-1164.

Zhang C, Wohlhueter R, Zhang H. 2016. Genetically modified foods: A critical review of their promise and problems. *Food Sci Hum Wellness* 5, 116-123.

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ORIGINAL ARTICLE

Prevalence and antimicrobial sensitivity of *Escherichia coli* and *Salmonella* species in field cases of rabbit intestinal coccidiosis treated with prebiotic

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ABSTRACT. Coccidian infection may promote the proliferation of gut bacteria of the family Enterobacteriaceae. Bacterial infections by members of this family in rabbits can induce a negative impact on their health and lead to high mortality, especially in young individuals. This study aimed to investigate the effect of prebiotic treatment on *Escherichia coli* and *Salmonella* species during natural intestinal coccidiosis in rabbits. Forty-five rabbits were selected from a rabbit farm in Beni-Suef, Egypt. Out of the 45 rabbits, 15 were coccidia-free and used as the negative control group (NC group) and 30 were naturally infected with coccidia. The infected rabbits were randomly divided into two equal groups, the positive control group (PC) and the prebiotic treated group (PT) which was orally treated with prebiotic for 8 successive days. Faecal oocyst count was assessed daily during the course of treatment. At 8 days post-treatment, 5 rabbits from each group were euthanised for the microbiological examination of the intestinal tract. On day 8 post-treatment, the PT group showed a significant ($P \leq 0.05$) reduction in the oocyst count ($5.33 \times 10^3 \pm 0.89$) with a significant ($P \leq 0.05$) decline in the prevalence of *E. coli* and *Salmonella* (66.7 % and 26.4%, respectively). The PC group showed highly a significant oocyst count ($21.67 \times 10^3 \pm 0.82$ OPG), with a significant increase in the prevalence of *E. coli* and *Salmonella* (86.7 % and 46.7 %, respectively). The NC group remained coccidian free and exhibited only *E. coli* with no *Salmonella* infection. The *in vitro* susceptibility test showed that *E. coli* isolates were highly resistant to most of the tested antimicrobials while *Salmonella* isolates showed variable resistance profiles. In conclusion, the prebiotic treatment significantly reduced the prevalence of *E. coli* and *Salmonella* infections coexisted with intestinal coccidiosis naturally infected rabbits.

Key words: rabbits, prebiotic, coccidiosis, *Salmonella*, *E. coli*, antimicrobial sensitivity.

INTRODUCTION

Rabbit production is a fast growing livestock industry worldwide. Rabbit meat is considered a source of animal protein that can solve the problem of the red meat shortage all over the world (Dalle and Szendro 2011). Digestive infections represent one of the main pathological problems and are responsible for significant economic losses in rabbit breeding facilities (Saravia *et al* 2017). Domestic rabbits are susceptible to a number of infectious diseases: parasitic, bacterial and viral (Langan *et al* 2000, Lennox and Kelleher 2009).

Rabbit coccidiosis is a disease caused by protozoan parasites of the genus *Eimeria* (Apicomplexa: Eimeriidae) (Pakandl 2009). Coccidiosis is mainly detected in young rabbits aged one to three months, especially after weaning, and it causes enteritis and diarrhoea and in severe cases infection may lead to death (Pakandl and Hlaskova 2007, Pakandl 2009, Papeschi *et al* 2013, El-Ashram *et al* 2019). Previous literature reported that coccidiosis has been associated with secondary bacterial and viral infections, which were common causes of mortality (Taylor *et al* 2003, Dorota *et al* 2012, Aboelhadid *et al* 2021). Rashwan and Marai (2000), Bortoluzzi et al (2019) and Madlala *et al* (2021) postulated that the coccidian infection may enhance the proliferation of Gram-negative bacteria of the family Enterobacteriaceae in the gut. Bacterial infections in rabbits can negatively affect the body condition and cause high mortality, especially at young ages (Zahraei *et al* 2010).

Escherichia coli (*E. coli*) is a common commensal bacterium of the gastrointestinal tract of warm-blooded animals. However, in an immune-suppressed host, certain *E. coli* strains become virulent and cause diarrheal and extraintestinal diseases (Croxen *et al* 2013). Hamed *et al* (2013) reported hemorrhagic colitis and diarrhoea in newborn New Zealand rabbits infected with *E. coli.* Also, *Salmonella enterica* infection in rabbits is considered a potential risk associated with this animal species (Suelam and Reda 2015). Its occurrence among domestic rabbits is probably variable (Rodriguez-Calleja *et al* 2006). According to Lim *et al* (2012), the prevalence of *Salmonella* species in rabbits ranged from 6 to 9%. It can occasionally cause a severe disease condition with a high mortality rate (Suelam and Reda 2015).

Antimicrobial therapy is one of the worldwide primary controls for the reduction of both incidence and mortality associated with bacterial diseases including colibacillosis and Salmonellosis in poultry (Hassan *et al* 2018, Radwan *et al* 2021). *In-vitro* antimicrobial susceptibility tests

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provide valuable guidance in the choice of appropriate drug treatment (Radwan *et al* 2021) and are very useful for detecting the MDR isolates. Recently, the phenomenon of antimicrobial resistance has evolved and spread out in large geographic areas. Therefore, the appropriate antimicrobials should be selected based on their susceptibility which could be detected by laboratory examination. Also, the challenge of antimicrobial resistance has increased the importance of searching for new compounds as alternative antimicrobials. Therefore, considerable effort has been devoted to investigating natural products to discover and develop new antimicrobial agents that are effective, easily accessible and affordable, with less side effects.

Prebiotics are non-digestible food components that promote the growth of beneficial bacteria in the digestive system and the host defence against infections (El-Abasy 2002, Sohail *et al* 2012). It was also observed that prebiotic consumption reduced the establishment of *Salmonella* in the course of hen moulting (Donalson *et al* 2008). Abdelhady and El-Abasy (2015) found that dietary supplementation of prebiotic and probiotic reduced mortality and overcame the adverse clinical signs in rabbits experimentally infected with *Pasteurella multocida*. Bio-Mos®, a prebiotic used in the animal husbandry industry, exhibited a suppressing effect on enteric pathogens and modulated the immune response of chickens and turkeys (Waldroup *et al* 2003, Kocher *et al* 2005). Mannan-oligosaccharide is now widely accepted as one of the most effective alternatives to antibiotics and growth promoters (Ferket *et al* 2002).

The present study aimed to investigate the effects of mannan-oligosaccharide (MOS) prebiotic supplementation on the coexistence of *E. coli* and *Salmonella* species in rabbits naturally infected with intestinal coccidiosis.

MATERIAL AND METHODS

The study was carried out on a rabbit farm at Sedes Station of Animal Production and Agriculture Research, Beni-Suef, Egypt. A total of 45 V-Line breed weaned rabbits, male and female, aged 30 to 35 days were selected for the experiment. Out of the 45 rabbits, 30 were naturally infected with intestinal coccidiosis (with the same average of oocyst count) and 15 were coccidian free. The infected rabbits showed the clinical signs of intestinal coccidiosis, e.g. diarrhoea, inappetence, abdominal bloating, and weight loss. These signs of coccidiosis were confirmed by detection and counting the oocysts in faeces by McMaster slide. All rabbits were examined individually and rabbits with oocyst count over 2.5×10^4 oocysts per gram faeces (OPG) were considered diseased according to Ogolla *et al* (2018). Each rabbit was housed in an individual wire mesh cage with optimum conditions and fed *ad libitum* with commercial rabbit feed (anticoccidial free). The clinically infected rabbits (n=30) were divided into two groups of 15 rabbits each; the first group was kept with no treatment and served as the positive control group (PC) while the second group was treated with prebiotic and served as the prebiotic treated group (PT) at a dose of 2 g/L for 8 successive days. The uninfected rabbits (n=15) served as the negative control group (NC). On day 8 post treatment, 5 rabbits from each group were humanely slaughtered for microbiology examination. The rabbits were handled and euthanised with the least distressful method which was cervical dislocation because they were not heavy weight (Walsh *et al* 2017). Death was verified by lack of breathing, lack of palpable heartbeat and fixed dilated pupil.

The study was approved by the ethical committee of Beni-Suef University (BSU- 0365/2018), Egypt. The used prebiotic was mannan-oligosaccharide (MOS) which derived from the cell wall of *Saccharomyces cerevisiae* (Bio-Mos®, Alltech, Nicholasville, USA).

FAECAL SAMPLE COLLECTION AND ESTIMATION OF OOCYST COUNT

Fresh faeces of each rabbit were collected daily during the course of treatment in all groups and the faecal oocyst count was estimated per gram of faeces using McMaster chamber according to Schito *et al* (1996). In brief, faecal pellets were weighed and diluted 10- fold in 2.5% potassium dichromate (w/v). For oocyst flotation, the mixture was vortexed and diluted in saturated sodium chloride. Finally, 300-µl of this dilution was loaded into the McMaster chamber, where the oocysts were allowed to float free of debris for 3-5 minutes before being counted. The diagnosis of different encountered *Eimeria* species was based on the descriptions mentioned by Eckert *et al* (1995). The following coccidian species were confirmed in the infected rabbits; *E. media, E. magna, E. intestinalis, E. flavescens and E. perforans.*

INTESTINAL SAMPLES AND BACTERIOLOGICAL EXAMINATION

Samples. Intestinal swabs were collected from the jejunum, ileum, and cecum of all euthanised rabbits (15 in total, 5 from each group). Accordingly, a total of 45 intestinal swab samples (15 from each group) were collected aseptically for bacteriological examination and screening of the presence of *E. coli* and *Salmonella* spp. The collected samples were transferred promptly, in an ice tank, to the microbiology laboratory.

Bacteriological isolation. Escherichia coli was isolated according to the protocol described by Radwan *et al* (2021). The collected samples were aseptically inoculated into MacConkey's broth and incubated aerobically at 37˚C for 24 hrs. Then, a loopful of each broth cultures was streaked onto tryptone soy agar and MacConkey's agar and incubated aerobically at 37˚C for 24-48hr. The lactose fermenting (pink) colonies were inoculated onto

eosin methylene blue (EMB) agar medium and incubated at 37˚C for 18-24 hrs.

Salmonella was isolated according to the protocol described by Hassan *et al* (2018). The collected samples were inoculated into selenite-F broth and incubated at 37C for 18-24 hrs. Then, a loopful of each culture was streaked out onto MacConkey's agar then the non-lactose fermenter (pale) colonies were streaked onto xylose lysine deoxycholate (XLD) and Salmonella-Shigella (SS) agar media and incubated at 37C for 18-24 hrs.

IDENTIFICATION OF *E. coli* AND *Salmonella* ISOLATES

Morphological and biochemical identification. All the recovered isolates were identified by microscopic examination of Gram's stained smears, colonial morphology and biochemical tests according to Collee *et al* (1996) and Quinn et al. (2002) using the following tests; oxidase, catalase, urease, H_2S production on TSI, and citrate utilisation. Moreover, a motility test in semisolid agar was applied.

Identification by using API20E kit. Escherichia coli and *Salmonella* isolates were also confirmed biochemically by using the API 20E system (BioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. Bacterial strains fully identified by the Department of Bacteriology, Mycology, and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt were used as a positive control in API kits.

SEROLOGICAL IDENTIFICATION OF BACTERIAL ISOLATES

Serogrouping of E. coli isolates. Escherichia coli isolates were serogrouped by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera according to Quinn *et al* (2002).

Serotyping of Salmonella. Salmonella isolates were serotyped by slide agglutination test using diagnostic polyvalent and monovalent O and H *Salmonella* antisera following the Kauffman-White- Le Minor scheme (Grimont and Weill 2007).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

All *E. coli* and *Salmonella* isolates were tested for their antimicrobial susceptibility to 14 different antimicrobial discs (Oxoid, Basing Stoke, UK) including; amoxicillin (10 µg), neomycin (15µg), tetracycline (30µg), sulphamazole-trimethoprim (25µg), nalidixic acid (30 µg), gentamicin (10µg), levofloxacin (5µg), florfenicol (30µg), colistin sulphate (10µg), ciprofloxacin (5µg), amikacin (30µg) and flumequine (25µg). An antimicrobial susceptibility test was applied using the disc diffusion method on Muller Hinton agar according to CLSI (2016). The antimicrobial susceptibility was based on the induced inhibition zones according to the guidelines of the CLSI (2016). Resistance to three/or more antimicrobials of different categories was taken as multidrug resistance (MDR) according to Chandran *et al* (2008).

STATISTICS

The data were summarised using a descriptive frequency and percentage for quantitative values. The relationship between data were tested by the Chi-square test for quantitative variables, and *p*-values were calculated. The oocysts per gram of faeces (OPG) were statistically analysed using ANOVA tests and subsequent Duncan's multiple range tests. Results were expressed as means ± SE. Probability of values less than 0.05 ($P \leq 0.05$) was considered significant.

RESULTS

FAECAL OOCYST COUNT AND PREVALENCE OF *E. coli* AND *Salmonella* ISOLATIONS IN DIFFERENT GROUPS

Faecal oocyst count revealed a gradual reduction from day zero (day of treatment) until day 8 post treatment in PC and PT groups. There was a significant ($P \leq 0.05$) decrease in oocyst count ($5.33 \pm 0.89 \times 10^3$) in rabbits treated with prebiotic (PT) when compared with those non-treated (PC) ($21.67 \pm 0.82 \times 10^3$) (figure 1). Meanwhile, the NC group revealed no oocysts in the faeces (table 1). During the period of treatment, the clinical signs were less severe

Table 1. Prevalence of *E. coli* and Salmonellae isolation in the examined rabbit samples in different groups.

Group	No. of samples	*E. coli* isolation		*Salmonella* isolation		X^2 (df)	P*
		No.	%	No.	%	22.003 (2)	0.000
Negative control (NC)	15	7	46.7	0	0		
Positive control (PC)	15	13	86.7	7	46. 7		
Prebiotic treated (PT)	15	10	66.7	4	26.7		
Total	45	30	66.7	11	24.4		

%: was calculated according to the number (No.) of tested isolates (n=30).
X^2: Chi-square. df: degree of freedom. * $P \leq 0.05$ is significant.

in the PT group than in the PC group. Rabbits in the PT group suffered from watery diarrhoea. However, rabbits in the PC group displayed diarrhoea with mucus, bloating, inappetence, and dullness. The rabbits in the NC group appeared normal with no clinical signs of disease.

On the other hand, the overall prevalence of *E. coli* and *Salmonella* isolates was 66.7% and 24.4%, respectively (table 2). The results showed a significant reduction in the prevalence ($P \leq 0.05$) of *E. coli* and *Salmonellae* isolates in rabbits of the PT group when compared with those of the PC group. In the PC group, 13 *E. coli* and 7 *Salmonellae* isolates were recovered with a prevalence of 86.7% and 46.7%, respectively. Meanwhile, 10 *E. coli* and 4 *Salmonellae* isolates were detected in the PT group with a prevalence of 66.7% and 26.7%, respectively. Regarding the NC group, only 7 *E. coli* isolates with a prevalence of 46.7% were found with no detection of any *Salmonellae*.

SEROLOGICAL IDENTIFICATION OF BACTERIAL ISOLATES

Serogrouping of E. coli isolates and their distribution in different organs. Out of 30 *E. coli* isolates, 7 O-serogroups were identified and O_{78} was the most prevalent representing 36.7% (table 3). The distribution of *E. coli* serogroups (n=30) in the different organs in all groups is shown in table 4.

Serotyping of Salmonella isolates and their distribution in different organs. Out of 11 *Salmonella* isolates, 3 *Salmonella enterica* Subsp. enterica serotypes were identified. The serotype *S. Macclesfield* was the most prevalent with 5 isolates representing 45.5% (table 5). The distribution of *Salmonella enterica* serotypes (n=11) in different organs in all groups is shown in table 5. No *Salmonella* isolates were recovered from rabbits in the NC group.

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *E. coli* AND *Salmonellae* RECOVERED FROM THE RABBITS INTESTINAL SAMPLES

Results of *in vitro* susceptibility testing showed that *E. coli* isolates were highly resistant to most of the tested antimicrobials and completely resistant to neomycin (100%) (table 6). Meanwhile, moderate sensitivities were recorded against colistin sulphate (50%) followed by ciprofloxacin (43.3%) and amikacin (40%) (table 6). MDR was detected in 27 *E. coli* isolates (90%).

On the other hand, *Salmonella* isolates (n=11) were highly resistant to sulphamethoxazol-trimethoprim and

Table 2. Serogroups of *E. coli* recovered from the intestinal tissue samples of the examined rabbits.

E. coli serogroups	No. of isolates	%
O_{78}	11	36.7
O_{125}	6	20
O_{152}	4	13.3
O_{158}	3	10
O_{114}	2	6.7
O_{115}	2	6.7
O_{168}	2	6.7
Total	30	100

%: was calculated according to the total number (No.) of isolates (n=30).

Table 4. Serotypes of *Salmonellae* recovered from the intestinal tissue samples of the examined rabbits.

Salmonella serotypes	No. of isolates	%
S. Macclesfield	5	45.5
S. Canada	3	27.3
S. Kisangani	3	27.3
Total	11	100

%: was calculated according to the total number (No.) of isolates (n=11).

Table 3. Distribution of *E. coli* serogroups in different organs in different groups.

Group	*E. coli* serogroups	Organ of isolation	No. of isolates	%
NC	O_{152}	Ilium	4	13.3
	O_{115}	Caecum	2	6.7
	O_{125}	Jejunum, Ilium and caecum	1	3.3
PC	O_{78}	Jejunum and Ilium	7	23.3
	O_{158}	Jejunum	3	10
	O_{125}	Jejunum, Ilium and caecum	2	6.7
	O_{114}	Jejunum	1	3.3
PT	O_{78}	Jejunum and Ilium	4	13.3
	O_{125}	Jejunum, Ilium and caecum	3	10
	O_{168}	Caecum	2	6.7
	O_{114}	Jejunum	1	3.3
Total			30	100

%: was calculated according to the total number (No.) of isolates (n=30).

Table 5. Distribution of *Salmonella* serotypes in different organs in different groups.

Group	*Salmonella* serotypes	Organ of isolation	No. of isolates	%
NC	Negative	Negative	Negative	Negative
PC	*S.* Kisangani	Ilium	3	27.3
	S. Macclesfield	Caecum	2	18.2
	S. Canada	Ilium, Jejunum	2	18.2
PT	*S.* Macclesfield	Caecum	3	27.3
	S. Canada	Ilium, Jejunum	1	9.1
Total			11	100

%: was calculated according to the total number (No.) of isolates (n=11).

Table 6. Antimicrobial susceptibility testing of *E. coli* and Salmonellae recovered from the intestinal tissue samples of the examined rabbits.

Antimicrobial disc	Disc content (µg)	*E. coli* (n=30)						*Salmonella* (n=11)					
		R		I		S		R		I		S	
		No	%	No	No	%	No	No	%	No	%	No	%
Amoxicillin	10	20	66.7	7	23.3	3	10	8	72.7	3	27.3	0	0
Neomycin	15	30	100	0	0	0	0	3	27.3	8	72.7	0	0
Tetracycline	30	23	76.7	5	16.7	2	6.7	4	36.4	5	45.5	2	18.2
Sulfamethoxazole-trimethoprim	25	24	80	6	20	0	0	9	81.8	1	9.1	1	9.1
Nalidixic acid	30	20	66.7	7	23.3	3	10	7	63.6	4	36.4	0	0
Gentamicin	10	14	46.7	9	30	7	23.3	1	9.1	3	27.3	7	63.6
Levofloxacin	5	12	40	9	30	9	30	0	0	5	45.5	6	54.5
Florophenicol	30	25	83.3	4	13.3	1	3.3	9	81.8	2	18.2	0	0
Colistin sulphate	10	15	50	0	0	15	50	2	18.2	1	9.1	8	72.7
Ciprofloxacin	5	10	33.3	7	23.3	13	43.3	0	0	2	18.2	9	81.8
Amikacin	30	9	30	9	30	12	40	4	36.4	4	36.4	3	27.3
Flumequine	25	24	80	5	16.7	1	3.3	8	72.7	3	27.3	0	0

%: was calculated according to the corresponding number of the tested isolates.

florophenicol (81.2% for each) (table 6). Meanwhile, high sensitivity to ciprofloxacin (81.8%) was recorded (table 6). MDR was detected in 5 *Salmonella* isolates (45.5%).

DISCUSSION

Rabbits are highly susceptible to enteric pathogens mainly in the early days after weaning and that may be due to the unestablished intestinal microbiota and ill-developed digestive performance and the change in gut PH (Pakandl 2009).

Prebiotics are food ingredients that induce the growth or activity of beneficial microorganisms such as bacteria and fungi (Gibson *et al* 2010). Also, prebiotics and probiotics are well known for modulation of the immune system and improvement of gut health (Hess and Greenberg 2012, Raheel *et al* 2019). Therefore, the current study was planned to investigate the effects of mannan-oligosaccharide (MOS) supplementation on the coexistence of *E. coli* and *Salmonella* species in rabbits naturally infected with intestinal coccidiosis.

In the present work, at the 8th day post treatment with mannan-oligosaccharide (MOS), there was a significant ($P \leq 0.05$) decrease in both oocyst count ($5.33 \pm 0.89 \times 10^3$) prevalence of *E. coli* and *Salmonella* infections (66.7% and 26.7%, respectively) in rabbits treated with prebiotic (PT). Meanwhile, the infected untreated group (PC) displayed a significant increase in both oocyst count ($21.67 \pm 0.82 \times 10^3$) and the prevalence of *E. coli* and *Salmonella* infections (86.7% and 46.7%, respectively). On contrary, the negative control group (NC) displayed only *E. coli* infection with a prevalence of 46.7% with no detection for coccidian oocysts and *Salmonella*. Also, the clinical signs of intestinal coccidiosis were less severe in the PT group

than in the PC group. These results are consistent with Aboelhadid *et al* (2021) who found that the use of prebiotic as prophylaxis significantly reduced the prevalence of the *E. coli* and *salmonella* infection in rabbits experimentally infected intestinal coccidiosis and diminished the coccidian adverse effect. Pakandl (2009) reported that enteritis caused by coccidia is often accompanied by a marked increase in the number of *E. coli* and other pathogens in the host intestine and hence the interplay between pathogens may be important under field conditions. Similar findings were reported by Kimura *et al* (1976) as they found an increase in the number of Enterobacteriaceae members during caecal coccidiosis in chicken. Also, Baba *et al* (1992) suggested that infection with *E. tenella* can change the balance of competitive adherence of bacteria, allowing more colonisation of *S. Typhimurium* and *Clostridium perfringens*. Additionally, Taylor *et al* (2003) recorded a significant secondary bacterial infection following coccidian infection and subsequently the malabsorption syndrome caused damage to the intestinal mucosa.

MOS prebiotic was derived from the cell wall of *Saccharomyces cerevisiae* which have the ability to improve gastrointestinal health and performance when added to animal diets (Kocher *et al* 2004, Miguel *et al* 2004). Similarly, we found a significant reduction in the prevalence of *E. coli* and *Salmonella* in rabbits naturally infected with intestinal coccidiosis, with amelioration in the adverse effects of coccidiosis after administration of MOS prebiotic. Also, Szabóová *et al* (2012) observed a significant reduction in bacterial and *Eimeria* oocyst counts in the intestinal tract of rabbits administered a mixture of prebiotic and probiotic as dietary supplementation. In addition, El-Ashram *et al* (2019) found a significant reduction in the adverse effects of intestinal coccidiosis in rabbits after prebiotic supplementation. Brink *et al* (2006) found that the growth of many Gram-positive and Gram-negative bacteria was inhibited by the prebiotic treatment. Also, Tran *et al* (2018) demonstrated that prebiotic supplementation can inhibit enteropathogens such as *Salmonella* and *E. coli*. Interestingly, Murate *et al* (2015) realised that the prebiotic additive reduced the occurrence of *Salmonella* in laying hens but not for broilers. This means that the effects of prebiotic are not constant and more studies are needed to report longitudinal load measure for establishing that prebiotic treatment can decrease the prevalence of *E. coli* and/or *Salmonella*.

The serogrouping studies of *E. coli* isolates (n=30) revealed that the serogroup O_{78} was the most predominant with a prevalence of 36.7%, followed by serogroups O_{1125}, O_{152}, O_{158} with a prevalence of 20%, 13.3% and 10%, respectively, and then the serogroups O_{114}, O_{115} and O_{168} with a prevalence of 6.7% for each. These results were similar to those reported by Hassan and Abd Al Azeem (2009), Shahin *et al* (2011) and Hamed *et al* (2013).

In the current study, *E. coli* isolates were completely resistant to neomycin and highly resistant to most of the tested antimicrobials especially florophenicol, sulphamethoxazol-trimethoprim, flumequine, tetracycline, amoxicillin and nalidixic acid. Meanwhile, they were moderately sensitive to colistin sulphate, ciprofloxacin and amikacin. Regarding the high incidences of antimicrobial resistance of *E. coli* isolates in this study, these findings provided more support to the reports of many authors in Egypt (El-Shazly *et al* 2017, El-Seedy *et al* 2019, Radwan *et al* 2021) and worldwide (Makhol *et al* 2011, Xiaonan *et al* 2018). Therefore, no single antimicrobial drug was effective by 100% against *E. coli* isolates, which might be due to the development of resistance as a result of indiscriminate use of antimicrobials. Moreover, MDR was detected in 90% of *E. coli* isolates which is in agreement with Radwan *et al* (2014) as they recorded MDR in 90.4% of isolates.

On the other hand, *Salmonella* isolates were highly resistant to sulphamethoxazol-trimethoprim, florophenicol, amoxicillin, flumequine and nalidixic acid and MDR was detected in 45.5% of the tested isolates. Meanwhile, it revealed high sensitivities against ciprofloxacin, colistin sulphate, gentamicin and levofloxacin. Similar findings were recorded by many authors in Egypt (Ahmed *et al* 2009, Hassan *et al* 2018) and worldwide (Kumar *et al* 2009, Camarda *et al* 2012, Kim *et al* 2012, Albuquerque *et al* 2014, Agrawal *et al* 2016, Lamas *et al* 2016). Increasing the occurrence of MDR strains led to antibiotic treatment failure in both humans and animals with the transmission of antibiotic resistance to other bacteria (Suelam and Reda 2015).

In conclusion, the use of Mann prebiotic induced a significant reduction in the prevalence of *E. coli and Salmonella* and at the same time mitigated the adverse effect of coccidiosis in rabbits. *E. coli* isolates were highly resistant to most of the tested antimicrobials while *Salmonella* isolates showed variable resistance profiles. In addition, MDR was detected in 90% of *E. coli* isolates and 45.5% of *Salmonella* isolates.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Abdelhady DH, El-Abasy MA. 2015. Effect of prebiotic and probiotic on growth, immuno-hematological responses and biochemical parameters of infected rabbits with *Pasteurella multocida. Benha Vet Med J* 28, 40-51.

Aboelhadid SM, Hashem S, Abdel-Kafy E-S, Mahrous LN, Farghly EM, *et al*. 2021. Prebiotic supplementation effect on *Escherichia coli* and *Salmonella* species associated with experimentally induced intestinal coccidiosis in rabbits. *PeerJ* 9, e10714.

Agrawal R, Hirpurkar SD. 2016. Prevalence of *Salmonella* on exterior and interior of fertile eggs from vaccinated breeding hens of different breeds. *J Dairy Vet Anim Res* 3, 178-183.

Ahmed AM, Younis EEA, Ishida Y, Shimamoto T. 2009. Genetic basis of multidrug resistance in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from diarrheic calves in Egypt. *Acta Trop* 111, 144-149.

Albuquerque AH, Maciel WC, Souza Lopes E, Castro Teixeira RS, Salles RPR, *et al*. 2014. Presence of *Salmonella* in one day-old chicks from hatcheries in the Metropolitan Region of Fortaleza, Brazil. *Acta Sci Vet* 42, 12-22.

Baba E, Wakeshima H, Fukui K, Fukata T, Arakawa A. 1992. Adhesion of bacteria to the cecal mucosal surface of conventional and germ-free chickens infected with *Eimeria tenella*. *Am J Vet Res* 53, 194-197.

Bortoluzzi C, Vieira BS, Hofacre C, Applegate TJ. 2019. Effect of different challenge models to induce necrotic enteritis on the growth performance and intestinal microbiota of broiler chickens. *Poultry Sci* 98, 2800-2812.

Brink M, Todorov SD, Martin JH, Senekal M, Dicks LM. 2006. The effect of prebiotics on production of antimicrobial compounds, resistance to growth at low pH and in the presence of bile, and adhesion of probiotic cells to intestinal mucous. *J Appl Microbiol* 100, 813-820.

Camarda A, Pugliese N, Pupillo A, Circella E, Ricci M, *et al*. 2012. Molecular characterization and antimicrobial resistance of *Salmonella* Typhimurium from rabbit farms in Southern Italy. *World Rabbit Sci* 20, 185-194.

Chandran A, Hatha A, Varghese S, Sheeja MK. 2008. Prevalence of multiple drug resistant *E. coli* serotypes in a tropical estuary, India. *Microbes Environ* 23, 153-158.

CLSI, Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing. 26th ed. *CLSI supplement M100-S*, Wayne, USA.

Collee JG, Fraser AG, Marmion BP, Simmons A. 1996. *Mackie and MacCartney Practical Medical Microbiology*. 14th ed. Churchill Livingstone, New York, USA, Pp 131-151.

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, *et al*. 2013. Recent advances in understanding enteric pathogenic *Escherichia coli. Clin Microbiol Rev* 26, 822-880.

Dalle ZA, Szendro Z. 2011. The role of rabbit meat as functional food. *Meat Sci* 88, 319-331.

Dorota K, Paweł B, Paweł N, Jerzy K. 2012. Natural alternatives to coccidiostats in rabbit nutrition. *Ann Anim Sci* 12, 561-574.

Donalson LM, McReynolds JL, Kim WK, Chalova VI, Woodward CL, *et al.* 2008. The influence of a fructooligosaccharide prebiotic combined with alfalfa molt diets on the gastrointestinal tract fermentation, *Salmonella* enteri-tidis infection, and intestinal shedding in laying hens. *Poultry Sci* 87, 1253-1262.

Eckert J, Taylor M, Licois D, Coudert P, Catchpole J, *et al*. 1995. Identification of *Eimeria* and *Isospora* species and strains: Morphological and biological characteristics. In: Eckert J, Braun R, Shirley MW, Coudert P (eds). *Biotechnology. Guidelines on Techniques in Coccidiosis Research*. Office for Official Publications of the European Communities, Luxembourg. Pp 103-119.

El-Abasy MA. 2002. Studies on sugar cane extract for control of chicken diseases. PhD Dissertation, Tokyo University, Tokyo, Japan.

El-Ashram AS, Aboelhadid SM, Abdel-Kafy EM, Hashem SA, Mahrous LN, *et al*. 2019. Prophylactic and therapeutic efficacy of prebiotic supplementation against intestinal coccidiosis in rabbits. *Animals* 9, 965.

El-Ashram S, Aboelhadid SM, Abdel-Kafy EM, Hashem SA, Mahrous LN, *et al*. 2020. Investigation of pre- and post-weaning mortalities in rabbits bred in Egypt, with reference to parasitic and bacterial causes. *Animals* 10, 537.

El-Seedy FR, Abed AH, Wafaa MMH, Bosila AS, Mwafy A. 2019. Antimicrobial resistance and molecular characterization of pathogenic *E. coli* isolated from chickens. *J Vet Med Res* 26, 280-292.

El-Shazly DA, Nasef SA, Mahmoud, FF, Jonas D. 2017. Expanded spectrum β-lactamase producing *Escherichia coli* isolated from chickens with colibacillosis in Egypt. *Poult Sci* 96, 2375-2384.

Ferket PR, Parks CW, Grimes JL. 2002. Benefits of dietary antibiotic and mannano-oligosaccharide supplementation for poultry. *Proceeding of the Multi-State Poultry Meeting*, Eagan, Minnesota, USA.

Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, *et al*. 2010. Dietary prebiotics: current status and new definition. *Food Sci Technol Bull Func Foods* 7, 1-19.

Grimont PA, Weill FX. 2007. Antigenic formulae of the *Salmonella* serovars. 9th ed. World Health Organization Collaborating Centre for Reference Research on *Salmonella*, Institute Pasteur, Paris, France.

Hamed AM, Eid AAM, El-Bakrey RMM. 2013. A review of rabbit diseases in Egypt. *Wartazoa* 23, 185-194.

Hassan SA, Abd Al Azeem MW. 2009. Determination of virulence gene markers and antimicrobial resistance in *Escherichia coli* isolated from rabbit in Egypt. *Glob Vet* 3, 260-267.

Hassan WH, Abed AH, Thabet A, El Nady EAM. 2018. Genetic analysis of multidrug resistant Salmonella isolated from broiler chickens. *J Vet Med Res* 25, 121-131.

Hess JR, Greenberg NA. 2012. The Role of Nucleotides in the Immune and Gastrointestinal Systems Potential Clinical Applications. *Nutr Clin Prac* 27, 281-294.

Kim MS, Lim TH, Jang JH, Lee DH, Kim BY, *et al*. 2012. Prevalence and antimicrobial resistance of *Salmonella* species isolated from chicken meats produced by different integrated broiler operations in Korea. *Poult Sci* 91, 2370-2375.

Kimura N, Mimura F, Nishida S, Kobayashi A. 1976. Studies on the relationship between intestinal flora and cecal coccidiosis in chicken. *Poult Sci* 55, 1375-1383.

Kocher A, Spring P, Hooge D. 2004. Rabbits may respond positively to dietary MOS. *Feedstuffs* 76, 1-3.

Kocher A, Garcia, P, Tucker LA. 2005. Effects of Bio-Mos for laying hens 20-52 weeks under commercial conditions. *Proceedings of the Alltech's 21st Annual Symposium on Nutritional Biotechnologies in the Feed and Food Industries*, Lexington, KY, USA, Pp 316-317.

Kumar Y, Sharma A, Mani KA. 2009. High level of resistance to nalidixic acid in *Salmonella enterica* serovar Typhi in Central India. *J Infect Dev Ctries* 3, 467-469.

Lamas A, Fernandez-No IC, Miranda JM, Vazquez B, Cepeda A, *et al*. 2016. Prevalence, molecular characterization and antimicrobial resistance of *Salmonella* serovars isolated from northwestern Spanish broiler flocks (2011-2015). *Poult Sci* 95, 2097-2105.

Langan GP, Lohmiller JJ, Swing SP, Wardrip CL. 2000. Respiratory diseases of rodents and rabbits. *Vet Clin North Am Small Anim Pract* 30, 1309-1335.

Lennox AM, Kelleher S. 2009. Bacterial and parasitic diseases of rabbits. *Vet Clin North Am Exot Anim Pract* 12, 519-530.

Madlala T, Okpeku M, Adeleke MA. 2021. Understanding the interactions between *Eimeria* infection and gut microbiota, towards the control of chicken coccidiosis: a review. *Parasite* 28, 48.

Makhol BM, Habreh N, Sakural K. 2011. Antibiotic resistance of *E.coli* isolated from poultry in Syria. *Assiut Vet Med J* 57, 265-275.

Miguel JC, Rodriguez-Zas SL, Pettigrew JE. 2004. Efficacy of a mannan-oligosaccharide (Bio- Mos®) for improving nursery pig performance. *J Swine Health Prod* 12, 296-307.

Murate LS, Fernanda GP, de Almeida AM, Berchieri A Jr, Shimokomaki M 2015. Efficacy of prebiotics, probiotics and synbiotics on laying hens and broiler challenged with *Salmonella* Enteritidis. *J Poult Sci* 52, 52-56.

Ogolla KO, Gathumbi PK, Waruiru RM, Okumu PO, Chebet J, *et al*. 2018. Efficacy of sulphachloropyrazine, amprolium hydrochloride, trimethoprim-sulphamethoxazole, and diclazuril against experimental and natural rabbit coccidiosis. *J Vet Med 2018, 5402469.*

Pakandl M. 2009. Coccidia of rabbit: A review. *Folia Parasitologica* 56, 153-166.

Pakandl M, Hlaskova L. 2007. The reproduction of *Eimeria flavescens* and *Eimeria intestinalis* in suckling rabbits. *Parasitol Res* 101, 1435-1437.

Papeschi C, Fichi G, Perrucci S. 2013. Oocysts excretion of three intestinal *Eimeria* species in female rabbits. *World Rabbit Sci* 21, 77-83.

Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC. 2002. *Veterinary microbiology and microbial disease*. Blackwell Science, Oxford, UK.

Radwan IA, Hassan HS, Abd-Alwanis SA, Yahia MA. 2014. Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated from septicemic broiler chicken. *Int J Adv Res* 2, 867-874.

Radwan IA, Abd El-Halim MW, Abed AH. 2021. Molecular characterization of antimicrobial resistant *Escherichia coli* isolated from broiler chickens. *J Vet Med Res* 27, 128-142.

Raheel IAR, Orabi A, Salam SH, Abed AH, Fouad IA, *et al*. 2019. Immune-modulating effects of Aviboost® nucleotides on the intestinal epithelium of Broiler chickens. *Inter J Vet Sci* 8, 89-95.

Rashwan AA, Marai IFM. 2000. Mortality in young rabbits: a review. *World Rabbit Sci* 8, 111-124.

Saravia M, Segovia C, Valderrama K, Santander J. 2017. Colibacillosis in a New Zealand white rabbit (*Oryctolagus cuniculus*). *J Infect Dev Ctries* 11, 203-206.

Schito ML, Barta JR, Chobotar B. 1996. Comparison of four murine *Eimeria* species in immunocompetent and immunodeficient mice. *J Parasitol* 82, 255-262.

Shahin AM, Lebdah MA, Ali GRM. 2011. *Escherichia coli* as an etiological agent of mucoid enteropathy in rabbits. *Researcher* 3, 8-16.

Sohail M, Ijaz A, Yousaf M, Ashraf K, Zaneb H, *et al*. 2010. Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. *Poultry Sci* 89, 1934-1938.

Suelam IIA, Reda LM. 2015. Isolation and identification of *Salmonella enterica* Typhimurium from rabbits. *Egy J Rabbit Sci* 25, 137-148.

Szabóová R, Lauková A, Chrastinová l', Strompfová V, Monika SP, *et al*. 2012. Beneficial effect of plant extracts in rabbit husbandry. *Acta Vet Brno* 81, 245-250.

Taylor MA, Catchpole J, Marshall J, Marshall, RN, Hoeben D. 2003. Histopathological observations on the activity of diclazuril (Vecoxan) against the endogenous stages of *Eimeria* crandallis in sheep. *Vet Parasitol* 116, 305-314.

Tran THT, Everaert N, Bindelle J. 2018. Review on the effects of potential prebiotics on controlling intestinal enteropathogens *Salmonella* and *Escherichia coli* in pig production. *J Anim Physiol Anim Nutr (Berl)*102, 17-32.

Waldroup PW, Oviedo-Rondon EO, Fritts CA. 2003. Comparison of Bio-Mos® and antibiotic feeding programs in broiler diets containing copper sulfate. *Int J Poult Sci* 2, 28-31.

Walsh JL, Percival A, Turner PV. 2017. Efficacy of blunt force trauma, a novel mechani-cal cervical dislocation device, and a non-penetrating captive bolt device for on-farm euthanasia of pre-weaned kits, growers, and adult commercial meat rabbits. *Animals* 7, 100.

Xiaonan Z, Yang J, Ju Z, Chang W, Sun S. 2018. Molecular characterization of antimicrobial resistance in *Escherichia coli* from Rabbit farms in Taiwan, China. *BioMed Res Int* 2018, 8607647.

Zahraei T, Mahzouniehand M, Khaksar E. 2010. Detection of *Salmonella* serovars in zoo and pet reptiles, rabbits, and rodents in Iran by culture and PCR methods. *Comp Clin Pathol* 19, 199-202.

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SHORT COMMUNICATION

Estimation of genetic parameters for subclinical mastitis using a threshold model in first parity dairy cows under pasture-based systems of Los Ríos Region in Chile

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ABSTRACT. Somatic cell count (SCC) is an indirect measurement to estimate mammary gland health status. This trait provides information regarding the severity of the mammary tissue inflammation in each quarter. Milk samples coming from the farm milk storage vat containing 100,000 to 200,000 cell/mL are considered suspicious, while SCC over 200,000 cell/mL is an indication of subclinical mastitis. Chilean dairy processors penalise farmers monetarily when their bulk tank samples reach levels of 300,000 cell/mL SCC. The objective of this study was to quantify the additive genetic component of the liability of cows to reach the 300,000 cell/mL threshold. A data set containing the highest SCC test-day record of 10,528 first lactation cows from 15 commercial dairy farms of Los Ríos Region in southern Chile was analysed. The unknown continuous underlying susceptibility of each cow to reach the 300,000 SCC threshold was modelled as a function of a contemporary group formed by the herd, year, and calving season, the regression coefficient of the unknown underlying susceptibility value of a cow on her daily milk yield (MY) and the additive animal genetic effect. Bayesian inference and Gibbs sampling were used to estimate additive and residual variances. The average daily MY and SCC were 17.84±5.25 kg and 125,327±236,297 cell/mL, respectively. The estimated heritability varied from 0.03 to 0.22 and the average was 0.10±0.03. It is concluded that the genetic variability for the susceptibility to reach the 300,000 SCC threshold could be exploited to improve resistance to subclinical mastitis.

Key words: milk, subclinical mastitis, threshold model, heritability.

INTRODUCTION

Mammary gland health status is a crucial issue in dairy cows to yield a milk volume and quality that is needed to keep an economically feasible dairy operation. Mastitis in dairy cattle is the most prominent and economically significant disease and a major cause of economic losses for dairy farmers. These losses are a direct result of reduced milk yield (MY), death, premature culling, veterinary costs, lost milk due to antibiotic use, and low milk quality (Bravo 2009, Miglior *et al* 2017, Ruegg and Pantoja 2013, Kirsanova *et al* 2019). However, subclinical mastitis is the most prevalent type of intramammary infection. This alteration cannot be detected by visual observation of the udder or milk because both appear normal, therefore, it remains a hidden disease. Cows with subclinical mastitis are usually not detected nor treated, and consequently, their reduction in MY and milk quality causes the greatest economic loss on dairy farms (Kumari *et al* 2018). An increase in somatic cell count (SCC) is observed as the health of the mammary gland decreases, therefore, the prevalence of subclinical mastitis is reflected in the herd SCC.

Somatic cell count is a well-known alternative procedure used to estimate mammary gland health condition that provides information regarding the severity of the mammary tissue inflammation in each quarter, and milk samples can also come from the farm milk storage tank (Bravo 2009, Kirsanova *et al* 2019, Ruegg and Pantoja 2013). According to the International Dairy Federation (1997), Sharma *et al* (2011) and Ruegg and Pantoja (2013) milk samples containing 100,000 to 200,000 cell/mL are considered suspicious, while SCC over 200,000 cell/mL of milk is an indication of subclinical mastitis presence. Chilean legislation does not explicit an SCC legal limit, however, in the southern regions of the country and, due to their low-quality milk association, domestic dairy processors economically penalise dairy farmers when their milk bulk tanks SCC reach 300,000 cell/mL.

Somatic cell count was introduced into many milk recording programs in North America and Europe in the late 1970s, raising renewed interest in selection for mastitis resistance (Miglior *et al* 2017). Several milk-producing countries have in place programs to reduce mastitis incidence and one of the actions is the genetic selection to reduce SCC by including this trait in their breeding programs (National Mastitis Council 2013). Except for the Scandinavian countries, direct selection for clinical mastitis has not been accomplished. In countries without regulated systems for dairy cattle health recording, obtaining sufficient records of health events for genetic evaluation is an issue that has not been properly addressed (Miglior *et al* 2017). At the farm level, individual cases of diseases are not routinely recorded and, therefore, data is not readily available. Nevertheless, there is evidence that mastitis could be reduced by selecting against affected cows (Miglior *et al* 2017), despite its low heritability (López-Villalobos *et al* 2014, Lembeye *et al* 2016).

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According to the International Dairy Federation (1997), cows with SCC over 200,000 cell/mL can be regarded as having subclinical mastitis, which allows using milk recording data to indirectly record subclinical mastitis incidence. Subclinical mastitis and many diseases may be recorded as present or absent, creating binary data and linear statistical models assuming normal distribution are not well suited for analyses of this type of data (Uribe *et al* 1995, Kadarmideen *et al* 2000). Non-linear threshold models have been proven to be theoretically better to analyse binary data and estimate genetic parameters (Gianola and Foullley 1983, Harville and Mee 1984).

The objective of this study was to estimate the prevalence and genetic variability of subclinical mastitis as an indirect trait based on surpassing an arbitrary SCC threshold, using a non-linear threshold model in first lactation dairy cows of Los Ríos Region, Chile.

MATERIAL AND METHODS

A data set containing 97,683 monthly test-day records of 10,528 first parity cows was used in this study, and from the pedigree files only 2,350 ancestors without records were included. Out of the 10,528 phenotyped animals, 7,377 had both parents known (71%), 2,109 had one parent missing (20%) and 1,042 had both parents missing (9%). Out of the 2,350 unphenotyped animals, 434 had both parents known (19%), 276 had one parent missing (12%), and 1,640 had both parents missing (69%). The above implies that the number of animals with missing parents is important.

Data gathered contained information from 1996 to 2019 in 15 commercial dairy herds of Los Ríos Region in southern Chile. Cow's breed composition was predominantly Holstein Friesian although other dairy breeds and crosses are also part of the southern Chile dairy population, unfortunately, the exact breed identification was not available in the data set.

Regarding the age of calving, only heifers calving for the first time from 20.5 to 40 months of age were included in this study. Milk yield test-day records below five and above 35 kg of MY and below six and above 305 days of lactation were deleted from the data set. Within each cow, its test-day records were sorted by SCC and only the largest record was left in the data set, hence the final data set had a single record on any of the 10,528 cows included in the study. Although subclinical mastitis results when SCC is greater than 200,000 cell/mL, most Chilean raw milk payment schemes penalise dairy farmers when bulk tank SCC reaches 300,000 cell/mL. In this study, the cows with records above such level were assumed to have subclinical mastitis.

Records of the presence or absence of subclinical mastitis, as defined in this study, generate discrete data that follows a binomial distribution. Therefore, estimation of genetic parameters by the usual method for mixed linear models, which are traditionally used for continuous traits, is not appropriate. However, it can be argued that subclinical mastitis observations lie on one of two ordered categories (surpassing or not 300,000 SCC), and susceptibility of animals to reach this limit follows an underlying continuous normal distribution that is not observed. Only those animals which exceed some threshold of susceptibility show more than 300,000 SCC. The underlying continuous susceptibility is assumed to be affected by both genetic and environmental factors and each animal has a non-observable, hypothetical random variable similar to a measurable phenotype in a continuous trait.

In such a model, the classification of an animal in one category or another depends on a susceptibility threshold, which is also unknown. In a usual mixed linear model, the outcome vector contains the real observations of a particular continuous trait, in this case, this vector represents the unobserved values on the underlying normal continuous scale of susceptibility to subclinical mastitis. This outcome vector is not observed directly, all that we observe is the presence or absence of subclinical mastitis. Gianola and Foullley (1983), and Harville and Mee (1984) proposed a nonlinear set of equations that are solved iteratively for the values of the threshold and effects included in the model (fixed and random), for the analysis of categorical data based on a threshold model.

The univariate animal threshold model, used to analyse the underlying susceptibility to subclinical mastitis and estimate variance components, was:

$$y_{ijk} = \mu + HYS_i + b_{ijk}(M) + a_j + e_{ijk}$$

Where: y_{ijk} = is the unknown continuous underlying susceptibility value of the observation k made by cow j in the contemporary group i. μ = is the population mean. HYS_i = is the fixed effect of the contemporary group made by cows controlled in the same herd, year, and season. b_{ijk} = is the regression coefficient of the unknown underlying susceptibility value of cow j on her test-day milk yield. M = is the milk yield of the cow j. a_j = is the random animal additive genetic effect which follows a normal distribution with mean equal cero and a covariance structure equal to the additive genetic relationship matrix multiplied by de genetic variance (σ^2_a) ~N(0, Aσ^2_a). e_{ijk} = is the residual error ~N(0, Iσ^2_e).

In the given contemporary group (HYS_i) all cows tested in the same herd (15), year (24), and test-day season (3) were included. The test-day season had three levels, cows tested from March to June, July to October (spring), and November to February were levels one, two, and three, respectively.

To solve the threshold model and estimate variance components the RENUMF90 and THRGIBBSF90 software were used[1]. The THRGIBBSF90 software handles threshold

[1] Misztal I, Tsuruta S, Lourenco DAL, Masuda Y, Aguilar I. 2018.

Table 1. Number of cows (N), mean, standard deviation (SD), and minimum (Min) and maximum (Max) values for milk yield (MY) and somatic cell count (SCC) by subclinical mastitis status (SMS).

SMS	N	Trait	Mean	SD	Min	Max
Up to 300,000 cell/ml.	6,985	MY[1]	17.03	5.42	5.00	34.80
		SCC[2]	130,409	73.579	18,000	300,000
Above 300,000 cell/ml.	3,543	MY[1]	16.30	5.45	5.00	34.74
		SCC[2]	871,832	634,497	301,000	2,995,000
All cows	10,528	MY[1]	16.78	5.44	5.00	34.80
		SCC[2]	379,922	511,663	18,000	2,995,000

[1] = kg/day
[2] = cell/mL

models using Bayesian inference and Gibbs sampling (Gianola and Sorensen 2002, Misztal 2008). In Bayesian statistics, the posterior distribution of a random variable is given by a prior density function which is updated by the information contained in the data, given the other parameters of a particular model. The joint posterior distribution contains all information needed to make inference about all parameters in the model, however, analytical integration of the joint posterior distribution, to obtain the marginal posterior distribution of the parameters of interest (covariances) is extremely difficult to perform in practice, therefore, approximations like the Gibbs sampler have been advocated to fully exploit Bayesian inference (Casella and George 1992). Gibbs sampling is an iterative process to draw the joint posterior distribution out of the samples generated as random numbers based on information available at a specific point. In a single iterate, the Gibbs sampler solves the mixed model equations with the current variance components and adds a small random number (noise) to each solution, variance components are then estimated. This process is repeated many times and, after a burn-in period, the average of samples (posterior marginal mean) provides estimators of covariance components. The corresponding mean is the Bayesian estimated parameter and, the standard deviation of samples (SD) corresponds to the standard error of the estimated variance component in a frequentist approach.

In this study, a single chain length of 200,000 was generated and the first 30,000 iterates of the chain were discarded as the burn-in period. The remaining 170,000 iterates were used for estimation of means of the marginal posterior distribution of the variance components as described by Sorensen *et al* (1995). Heritability (h^2) of the unknown continuous underlying susceptibility to subclinical mastitis was estimated as $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$, where: σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance.

Manual for BLUPF90 family programs. University of Georgia. Available at: http://nce.ads.uga.edu/wiki/doku.php?id=documentation

RESULTS AND DISCUSSION

The average daily MY and SCC in this sample of 10,528 first lactation cows were 16.78±5.44 kg and 379,922±511,662 cell/mL, respectively. Minimum and maximum SCC were 18,000 and 2,995,000 cell/mL, respectively. These averages included only the highest SCC record of each cow, when all test-day records of each cow were considered (97,256) the corresponding averages were 17.84±5.25 kg of milk and 125,327±236,297 cell/mL, respectively. Assuming a 305 days lactation the estimated lactation MY is 5,441 kg. This value is lower than the average indicated by Montaldo *et al* (2015) who studied G×E interaction of proven sires between the US and Chile, analysed 243,134 Chilean cow lactations gathered from 1997 to 2008 and reported that the average lactation MY across lactation was 8,082 kg. This large difference can be explained because the lactation records used by Montaldo *et al* (2015) included all lactations and were adjusted to 305 days mature equivalent MY. Also, a MY higher (7,408 kg) than that reported in this research was that indicated by Pinedo and Meléndez (2010) for 305 days mature equivalent MY in Chilean Holstein cows. The literature reviewed in this study does not report the average test-day milk yield for Chilean cows.

The average SCC, calculated using all available records (97,256) of the 10,528 cows, was 125,327±236,297 cell/mL which is lower than the SCC reported by Pinedo and Meléndez (2010) who included lactations from 187 herds recorded from 1997 to 2007 and indicated that the average SCC decreased from 489,000 to 309,000 cell/mL. Similar to the present study, Werner (2014) reported an average SCC of 151,131 cell/mL by analysing 640,249 Chilean lactations in farms located in southern Chile (Malleco to Chiloé).

Table 1 shows the number of cows, mean, standard deviation, and minimum and maximum values for milk yield and SCC according to the subclinical mastitis status of the cows. Sixty-six per cent of the cows (6,985) did not reach the threshold of 300,000 cell/mL while the remaining 34% (3,543 cows) were classified as having subclinical mastitis. As expected, cows that reached the 300,000 cell/

mL SCC threshold yielded less milk (16.30±5.45 kg) when compared to healthy cows (17.02±5.42 kg).

Considering the highest SCC record of each cow used in this study (10,528 cows, table 1), the average SCC was 379,921 cell/mL which is greater than the average SCC of 151,131 cell/mL reported by Werner (2014). A possible explanation is that in this study, among all test-day records available for each cow only the greatest one was used in computing the average. Pineda and Meléndez (2010) reported an average SCC of 309,000 cell/mL in 2007, and according to the International Dairy Federation (1997) guidelines the average Chilean Holstein cow had subclinical mastitis, fortunately, this prevalence has decreased according to Werner (2014) who indicated an average SCC of 151,131 cell/mL.

Somatic cell count is an accepted indirect method for the diagnostic of subclinical mastitis (Bravo 2009, Kirsanova *et al* 2019, Ruegg and Pantoja 2013), and albeit the International Dairy Federation (1997) indicated that an SCC above 200,000 cell/mL is an indication of subclinical mastitis presence, in this study the SCC threshold was arbitrarily fixed at 300,000 cell/mL because this is the limit accepted by the Chilean dairy processors to start monetarily penalising raw milk. Other definitions of subclinical mastitis, based on the number of consecutive days reaching a given SCC threshold, have also been explored (Bobbo *et al* 2018). According to the definition of subclinical mastitis chosen in this study, thirty-four per cent of the cows had the disease and their average SCC was 871,832 cell/mL, while cows that did not reach the SCC threshold had an SCC average of 130,409 cell/mL (table 1). Bravo (2009) used data from 1,286 black and white dairy cattle of a research farm in Purranque, south of Chile, to estimate a subclinical mastitis prevalence of 38.9% which is similar to that reported in this study. Bobbo *et al* (2018) analysed 574,174 test-day records of 66,784 first parity Holstein cows (20 to 40 months of age) from 404 herds in northeast Italy and reported that subclinical mastitis infected cows, defined as those that reached an SCC of 400,000 cell/mL, was 47%.

Table 2 shows the means and their standard errors of 170,000 Gibbs samples of the marginal posterior distribution for genetic and residual variances. Figure 1 shows the sample values pattern of h^2 after the 30,000 samples burn-in period, the steadiness of the pattern indicates that convergence has been met. Similar steady patterns were also obtained for genetic and residual variances. All standard deviations were very low as compared to their

Table 2. Estimated mean, standard error (SE), minimum (Min) and maximum (Max) values of the genetic (σ_a^2) and residual (σ_e^2) variance, and heritability (h^2).

	Mean[1]	SE	Min	Max
σ_a^2	0.12	0.04	0.03	0.29
σ_e^2	1.04	0.02	0.96	1.13
h^2	0.10	0.03	0.03	0.22

[1] =170,000 Gibbs samples.

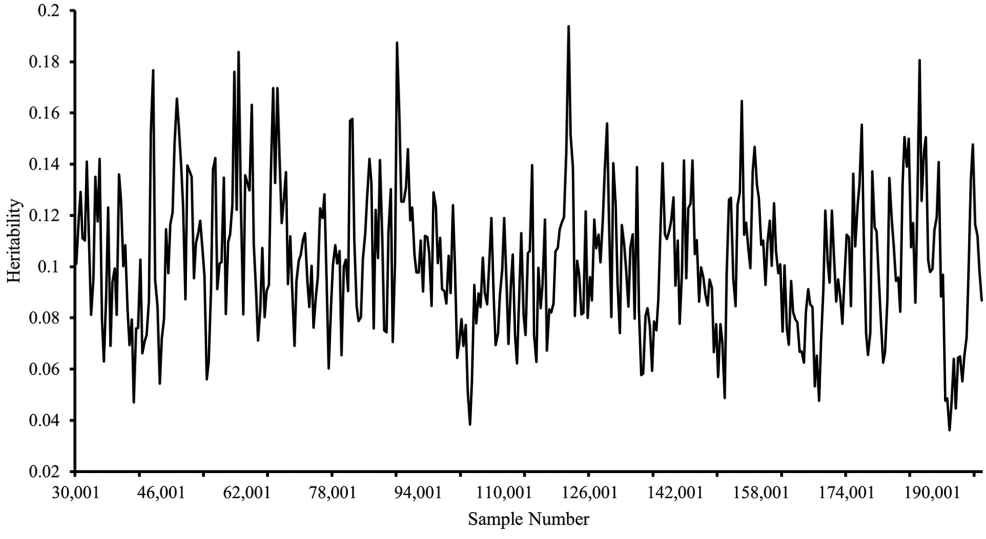

Figure 1. Gibbs samples pattern for heritability estimates after the burn-in period.

corresponding means which indicates that the estimated parameters are different from zero hence not meaningless.

Heritability estimated for subclinical mastitis in this study was 0.10±0.03 (table 2). Using a linear repeatability animal model Kirsanova *et al* (2019) estimated h^2 in Norwegian Red cows in lactation 1 to 3, for several subclinical mastitis traits as defined according to SCC thresholds from 50,000 to 400,000 cell/mL, their estimate for the 300,000 cell/mL threshold was 0.06±0.002 which is inferior to that reported in this study. In the study of Kirsanova *et al* (2019), smaller SCC thresholds had higher h^2 estimates, for instance, the 150,000 cell/mL threshold had h^2 equal to 0.10±0.002 which is identical to the h^2 estimated here for the 300,000 cell/mL threshold. Uribe *et al* (1995), estimating genetic parameters for common health disorders of Canadian Holstein cows and using a non-relationship sire threshold model, reported h^2 for clinical mastitis of 0.15. Kadarmideen *et al* (2000) estimated the h^2 of several clinical diseases in UK dairy cows and their estimation for clinical mastitis using a non-relationship sire threshold model was 0.126±0.033. On the other hand, Bobbo *et al* (2018) estimated h^2 of 0.06±0.01 for Italian Holstein cows reaching a SCC threshold of 400,000 cell/mL, this is lower than the h^2 estimated in this study and could be partially explained because Bobbo *et al* (2018) used a multiple trait linear model for a binary instead of a threshold model like the one used in this study.

A somatic cell count is a management tool and milk quality criterion which is incorporated in all milk recording schemes and can be used in genetic selection because of its association with both, clinical and subclinical mastitis (Sharma *et al* 2011). However, few studies have researched the genetic variability of alternative SCC traits. Nordic countries, where only veterinarians are allowed to treat animals, have nationwide systems for health data recording (Miglior *et al* 2017). In countries like Chile with no regulated systems in place for dairy cattle health recording and having a sound milk recording scheme, the use of alternative SCC traits can be used in genetic selection to increment mastitis resistance.

The results of this study are relevant since it is widely recognised that SCC is a trait economically important as an indicator for mastitis infection. In Chile, the first selection index for the Chilean dairy cattle, under pastoral systems, was developed (VEL; Valor Económico Lechero for its acronym in Spanish) (Lama and Vargas 2020). In this index, mammary health is one of the seven traits included. Since there are no previous studies on genetic parameters for mastitis resistance in the Chilean dairy cattle, our results can be used for simulation studies to predict genetic resistance to mastitis.

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REFERENCES

Bobbo T, Penasa M, Finocchiaro R, Visentin G, Cassandro M. 2018. Alternative somatic cell count traits exploitable in genetic selection for mastitis resistance in Italian Holsteins. *J Dairy Sci* 101, 10001-10010.

Bravo K. 2009. Estudio de incidencia y prevalencia de mastitis y su impacto económico en lecherías de la X región. 67 p. *Undergraduate Dissertation,* Universidad de Chile, Santiago, Chile.

Casella G, George EI. 1992. Explaining the Gibbs sampler. *Amer Stat* 46, 167-174.

Gianola D. Foulley JL. 1983. Sire evaluation for ordered categorical data with a threshold model. *Genet Sel Evol* 15, 201-224.

Gianola D, Sorensen D. 2002. *Likelihood, Bayesian and MCMC methods in quantitative genetics*. Springer-Verlag Inc., New York, USA.

Harville DA, Mee RW. 1984. A mixed model procedure for analyzing ordered categorical data. *Biometrics* 40, 303-408.

International Dairy Federation. 1997. Recommendations for presenting of mastitis related data. *IDF Bulletin 321,* Brussels, Belgium.

Kadarmideen HN, Thompson R, Simm G. 2000. Linear and threshold model genetic parameters for disease, fertility and milk production in dairy cattle. *Anim Sci* 71, 411-419.

Kirsanova E, Heringstad B, Lewandowska-Sabat A, Olsaker I. 2019. Alternative subclinical mastitis traits for genetic evaluation in dairy cattle. *J Dairy Sci* 102, 5323-5329.

Kumari T, Bhakat C, Choudhary RK. 2018. A review on sub clinical mastitis in dairy cattle. *Int J Pure App Biosci* 6, 1291-1299.

Lama J, Vargas P. 2020. Indice económico para la selección de bovinos lecheros en Chile. *Cooprinforma* 157, 18-21.

Lembeye F, López-Villalobos N, Burke JL, Davis S. 2016. Estimation of genetic parameters for milk traits in cows milked once- or twice-daily in New Zealand. *Livest Sci* 185, 142-147.

López-Villalobos N, Edwards JP, Jago JG. 2014. Estimation of genetic and crossbreeding parameters of milking characteristics of grazing dairy cows. *New Zealand J Agric Res* 57, 180-191.

Miglior F, Fleming A, Malchiodi F, Brito LF, Martin P, *et al*. 2017. A 100-year review: Identification and genetic selection of economically important traits in dairy cattle. *J Dairy Sci* 100, 10251-10271.

Misztal I. 2008. Reliable computing in estimation of variance components. *J Anim Breed Genet* 125, 363-370.

Montaldo HH, Castillo-Juárez H, Lizana C, Trejo C, Cienfuegos-Rivas EG, *et al*. 2015. Genotype-environmental interaction between Chile and North America and between Chilean herd environmental categories for milk yield traits in Black and White cattle. *Anim Sci Pap Rep* 33, 23-33.

National Mastitis Council. 2013. *Proceedings of the 52nd Annual Meeting*. NMC, San Diego, California.

Pinedo PJ, Meléndez P. 2010. Patrones temporales de recuento de células somáticas, grasa, proteína y nitrógeno ureico en leche de estanque y su asociación con fertilidad en ganado lechero en la zona centro-sur de Chile. *Arch Med Vet* 42, 41-48.

Ruegg PL, Pantoja JCF. 2013. Understanding and using somatic cell counts to improve milk quality. *Irish J Agric Food Res* 52, 101-117.

Sharma N, Singh NK, Bhadwal MS. 2011. Relationship of somatic cell count and mastitis: An Overview. *Asian-Aust J Anim Sci* 24, 429-438.

Sorensen DA, Andersen S, Gianola D, Korsgaard I. 1995. Bayesian inference in threshold models using Gibbs sampling. *Genet Sel Evol* 27, 229-249.

Uribe H, Kennedy BW, Martin SW, Kelton DF. 1995. Genetic parameters for common health disorders of Holstein cows. *J Dairy Sci* 78, 421-430.

Werner EG. 2014. Relación de la producción de leche y calidad sobre el recuento de células somáticas en rebaños del sur de Chile. *Undergraduate Dissertation*, Universidad Austral de Chile, Valdivia, Chile.

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SHORT COMMUNICATION

Assessment of the CPL-0015 isolate as a vaccine strain for the control of canine parvovirus in Cuba

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ABSTRACT. The safety and protective efficacy of the CPL-0015 Cuban isolate of canine parvovirus type 2 (CPV-2) were evaluated for its possible use as a vaccine strain candidate. The study included a total of 23 healthy Beagle dogs of both sexes, aged 84 days and without specific maternal antibodies against canine parvovirus. Safety was analysed by comparing clinicopathological values, food consumption, body weight, rectal temperature and white blood cell counts for 14 consecutive days between control dogs (n=5) and dogs subcutaneously injected (n=10) with 2 mL (equivalent to two doses) of the CPL-0015 strain with an antigenic titer of $10^{6.0}$ infectious $dose_{50}$ in cell culture/mL. The protective effectiveness was determined by measuring and comparing anti-CPV-2 IgG levels and clinical signs during 56 experimental days between control dogs (n=2) and dogs inoculated (n=6) with double doses of 1 mL each, separated by a 21-day interval. All animals were challenged orally on day 35 with the virulent strain Cornell-780916 ($10^{5.0}$ infective $dose_{50}$ in cell culture/mL). The results showed that the CPL-0015 strain did not negatively impact the physiological condition of the exposed animals. The inoculated and challenged animals showed not only significantly increased levels of anti-CPV-2 IgG ($P<0.05$) when compared to days 0, 35, and the control group animals but also had 100% survival without clinical signs of the disease, unlike the control group. It is concluded that CPL-0015 is safe and provides effective protection against homologous virulent strains.

Key words: canine parvovirus, dogs, safety, protection, vaccine.

INTRODUCTION

Canine parvovirus (CPV) is the cause of serious enteric infections in *Canis familiaris*, resulting in acute clinical progression with high morbidity and mortality regardless of age, race, or gender (Aponte *et al* 2020, Dong *et al* 2020). However, several authors consider that certain factors such as younger age, nonconfinement, and lack of vaccination play a key role in its spread, clinical course, and fatal outcome (Zhuang *et al* 2019, Qi *et al* 2020). CPV is characterised by its genetic diversity and alarming ability to cross the interspecies barrier, enabling it to adapt to multiple hosts, both domestic and wild (Miranda and Thompson 2016, Li *et al* 2017, Zhou *et al* 2017, Voorhees *et al* 2019).

The immunisation of domestic dogs with modified live vaccines containing the CPV-2 strain is an effective method of controlling and preventing the clinical development of the disease (Domínguez *et al* 2014, Mukthar *et al* 2021). However, in recent years the use of these formulations has become controversial due to their failure in protecting vaccinated animals (Decaro *et al* 2020, Ying *et al* 2020). Although the cause of these events is multifactorial (Altman *et al* 2017), much attention is being paid to the possible inability of the CPV-2 vaccine strain to induce protection against the new genetic variants CPV-2a, CPV-2b, and CPV-2c (Ying *et al* 2020). Such suspicions are supported by the antigenic variations detected in emerging lineages associated with several mutations located in the VP2 structural protein, an important antigenic determinant present in the viral capsid (Li *et al* 2017, Sebastian *et al* 2019, Ying *et al* 2020).

As a result, among other aspects, there is an urgent need to evaluate the immunological consequences of the genetic diversity of CPV, the genotypes prevailing in different countries, the antigenic properties of each one, and their possible vaccine potentialities (Zhou *et al* 2017, Sebastian *et al* 2019, Hao *et al* 2020). The antigenic variability observed at the global level does not seem to be present in Cuba. Molecular studies (results not presented) carried out after those published by Fresneda *et al* (2015) from diseased animals, demonstrated the circulation of the CPV-2 strain only. The existence in our archipelago of original pathogenic strains that persist over time is not exclusive to canine parvovirus, it was also reported for *Pasteurella multocida* subsp *multocida* biovar A:1 in rabbits (Lugo *et al* 2019, Domínguez *et al* 2021).

Recent Cuban epidemiological studies revealed that no racial dogs with free access to the street, the consumption of inadequate food including raw meat, and not vaccination are factors that increase the chances of becoming ill from CPV (Pino *et al* 2019, Peña *et al* 2020). In this context, having its own vaccine will make it possible not to depend on international suppliers of vaccines to control the disease. Besides, the possibility of achieving technological sovereignty would reduce the prices of the biological product and promote its commercialisation in the foreign market. This perspective led us to isolate, attenuate in cell culture, and characterise circulating strains native to Cuba (Fresneda *et al* 2015). Accordingly, a vaccine formulation

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was developed with our own technology, adapted to the Cuban epidemiological reality, independently of external strains. Consequently, the present study assessed the safety and protective efficacy of the Cuban isolate, CPL-0015, of canine parvovirus type 2 as a vaccine strain in Beagle dogs without specific maternal antibodies against parvovirus canine.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

This experiment followed national (GOC-2021-332-EX25) and institutional guidelines for the care and use of animals. All experimental procedures were approved by the Committee of Ethical Review at Grupo Empresarial LABIOFAM (protocol approval number: 06/20; experimental period: October 2020). The selection of the biomodel, age and number of individuals per experimental group took into account several existing international references on clinical evaluations of classic and modern vaccines against canine parvovirus (Langeveld *et al* 2001, Siedek *et al* 2011, Hernández *et al* 2015). The study included a total of 23 healthy Beagle dogs of both sexes, aged 84 days (12 weeks), with no history of vaccination, testing negative for maternal antibodies against canine parvovirus (absorbance <0.18) and free of bacterial (Salmomella spp., *Escherichia coli*, Leptospira spp., *Borrelia burgdorferi, Ehrlichia canis* and *Mycoplasma haemocanis*), viral (Canine parvovirus, Canine hepatitis virus, Canine distemper virus and Canine rabies) and parasite (Nematode spp., Cestode spp., Babessia spp. and Ectoparasite) specific diseases for the species. The animals were placed in separate cages and were provided with adequate food based on the species and age (three times a day) and free access to water. After the adaptation period (seven days), the animals were randomly distributed (15 for the safety study and 8 for the efficacy study).

IMMUNOGENS

Inoculum. The candidate vaccine strain CPL-0015 (CPV-2) was isolated in 1991, attenuated by 53 passes in Madin-Darby Canine Kidney cells (MDCK) and then stabilised through 27 passes in Crandell-Rees Feline Kidney Cell (CRFK) (Fresneda *et al* 2015). A vial of lyophilised CPL-0015 isolate (Batch 1203010) with a titer of $10^{6.0}$ tissue culture infectious dose $(TCID)_{50}$/mL was reconstituted with 1 mL of minimum essential medium. This volume was inoculated into the CRFK line supplemented by 2% fetal bovine serum. After incubating for seven days at 37ºC and observing the cytopathic effect in more than 80% of the culture, we proceeded to collect, freeze-dry, and preserve it at −70ºC. Before use, one of the obtained virals was diluted with water for injection to obtain a titer of $10^{6.0}$ $TCID_{50}$ in a total volume of 1 mL (figure 1).

Challenge strain. Over the base of previous international experiences (Cunegündes *et al* 2008, Oliveira *et al* 2010, De Cramer *et al* 2011, Puentes *et al* 2012), the worldwide virulent Cornell-780916 strain (type 2) from the collection ATCC with the number ATCC® VR- 2006™ was selected. Before its use, it was titled in the MDCK cellular line with a value of $10^{6.5}$ and then diluted with minimum essential media to obtain a titer of $10^{5.0}$ $TCID_{50}$ in a total volume of 1 mL/animal (figure 1).

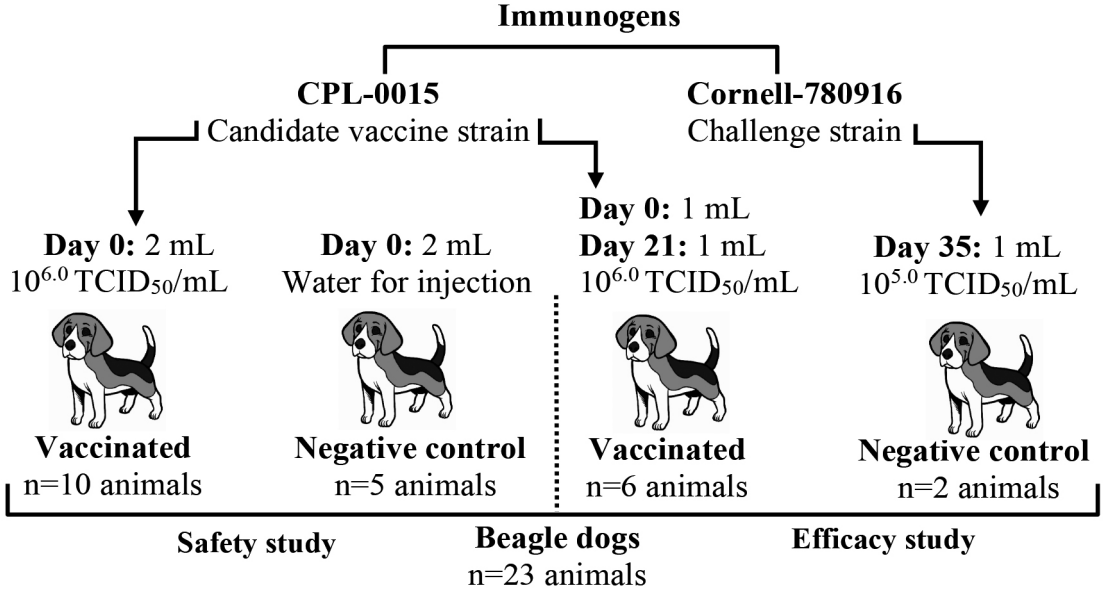

Figure 1. General aspects of the designed studies to evaluate the efficacy and safety of the CPL-0015 strain.

SAFETY STUDY

Two experimental groups were established (figure 1). One group was inoculated with CPL-0015 at a titer of $10^{6.0}$ $TCID_{50}$/mL (n=10), whereas the negative control group received water for injection (n=5). All animals received double doses (2 mL) subcutaneously in the dorsal area of the neck and were manipulated by the same researcher. The injection site was observed for 14 consecutive days to look for local alterations and the animals were monitored for general clinical manifestations or changes in the rectal temperature. Furthermore, white blood cell counts, food consumption, and body weight were assessed on alternate days.

A clinical assessment system was followed based on possible and typical disease alterations. Scores ranged from 1 to 9 depending on the severity of the clinical alterations (table 1). Euthanasia was performed using a sodium pentobarbital (Pentovet, 150 mg/kg, intraperitoneal) overdose when the score was greater than 7.

EFFICACY STUDY

The animals used in this study were divided into two experimental groups (figure 1). One group (n=6) was inoculated with two subcutaneous doses (1 mL each) of CPL-0015 ($10^{6.0}$ $TCID_{50}$) with an interval of 21 days between doses (days 0 and 21), whereas the negative control group (n=2) received water for injection in the same volume, frequency, and route of administration as those of the inoculated group. Fourteen days after the second injection (experimental day 35), all animals were challenged orally with the virulent Cornell strain. Clinical follow-up of all animals was performed from day 0 to experimental day 56 using the abovementioned clinical assessment system. The first 35 days, were dedicated to observe the possible adverse reactions of the CPL-0015 initial dose and the recollection, using the same written indicators previously described.

Table 1. Clinical assessment system used in the safety study.

Clinical score	Clinicopathological alterations
1	No symptoms
2	Pain at the injection site
3	Redness at the injection site
4	Unusual skin manifestations at the injection site (erythema, alopecia, etc.)
5	Fever + decreased willingness to eat and drink
6	Mucoid or hemorrhagic diarrhea
7	Vomiting + mucoid or hemorrhagic diarrhea
8	Anorexia + weakness + prostration
9	Death

HAEMATOLOGY AND SEROLOGY

Blood samples for assessing the white blood cell counts of the animals in the safety study were collected on experimental days 0, 2, 4, 6, 8, 10, 12, and 14. In all cases, 1 mL of blood was collected from the lateral saphenous vein into tubes containing an anticoagulant. Additionally, the evaluation of anti-CPV IgG antibody titers was performed on days 0, 35, and 56 using 2 mL of heat-inactivated serum (56ºC for 30 minutes). A solid phase, quantitative, indirect enzyme-linked immunosorbent assay system was used (CENPALAB, Cuba). The reading of the samples was taken at 492 nm, and the cut-off value was 0.18 according to the manufacturer's instructions.

STATISTICAL ANALYSIS

For the analysis of immune response variables, data from the inoculated and control groups were compared using the Wilcoxon-Mann-Whitney test. A value of $P<0.05$ was considered statistically significant. The statistical program used for the analyses was SPSS 12.0 (SPSS Inc, Chicago IL, USA).

RESULTS AND DISCUSSION

Vaccination and use of live attenuated vaccines manufactured using classic production technologies continue to be essential tools for the control of canine parvoviruses worldwide (Puentes 2012, Domínguez *et al* 2014, Mukthar *et al* 2021). Therefore, controlled clinical studies using autochthonous circulating strains adapted to cell culture are important in order to produce immunoprophylactic formulations adapted to current epidemiological situations.

SAFETY STUDY

Clinical observation for 14 consecutive days after the inoculation of an overdose of the Cuban isolate, CPL-0015, demonstrated the absence of undesirable local or systemic side effects. Analysis of the white blood cell count, rectal temperature, food consumption, and body weight showed that the inoculation of CPL-0015 did not have a negative impact on any animal under these conditions (data not shown). The negative control group, on the other hand, showed similar results and clinical scores (level 1) as those of the inoculated group.

The use of clinical indicators to detect the occurrence of negative events associated with immunoprophylactic formulations (Day 2008) and the evaluation of vaccine antigens (Moore and Hogen 2010) is well-known. In our case, the lack of adverse consequences in animals inoculated with a CPL-0015 overdose supports its suitability for use in puppies. This characteristic of CPL-0015 will be greatly beneficial if used in future formulations because although it is a living virus belonging to the family Parvoviridae, it

shows a high affinity for growing cells having a high rate of mitotic division (Díaz *et al* 2008).

EFFICACY STUDY

Figure 2 summarises the antibody response of the Beagle dogs from both the experimental and control groups at different time intervals. Prior to inoculation (day 0), anti-CPV antibodies were undetectable (values below 0.18) with the technique used, confirming the absence of prior immunological stimulation. On day 35, at the time of the challenge, all animals inoculated with CPL-0015 (days 0 and 21) showed a significant percent increase (490%) in their antibody levels compared to the control group animals who continued to be seronegative. On experimental day 56 (21 days after the challenge), although in general terms significant progressive increases in IgG levels were found in both groups ($P>0.05$), these were not homogeneous. Animals inoculated with CPL-0015 showed the greatest and most significant percent increases in their antibody levels not only with respect to days 0 and 35 (1,460% and 297%, respectively) but also with respect to the control group (129%) in the same period.

Furthermore, figure 3 shows the results in terms of clinical score for the experimental and control groups before and after the challenge. In animals inoculated with CPL-0015, there was an absence of adverse local or systemic reactions from days 0 to 14 after the second injection (experimental days 35) and signs of disease after the challenge. In contrast, unvaccinated animals challenged with the virulent Cornell-780916 strain began to show progressive clinical signs typical of CPV from experimental day 39 (day 4 after the challenge). Control animals shared the same score until day 13 after the challenge, when

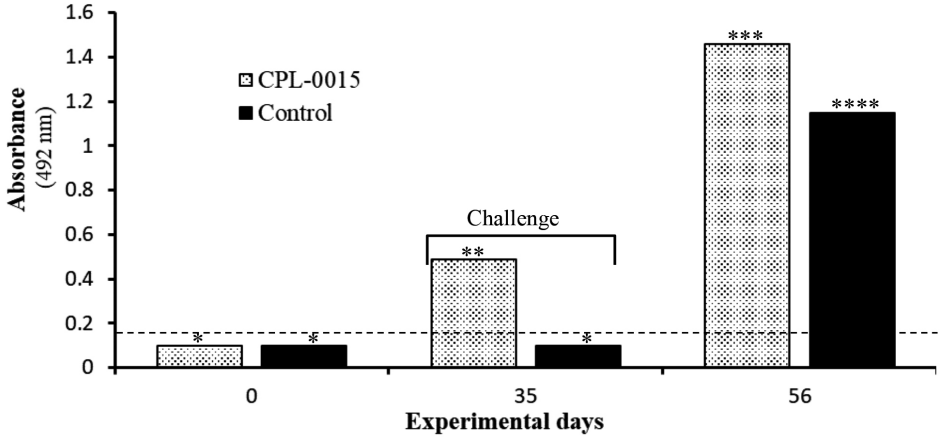

Figure 2. Immune response of Beagle dogs inoculated with strain CPL-0015 and controls.

Note: Different number of symbols indicate significant differences ($P<0.05$) based on the Wilcoxon-Mann-Whitney test.

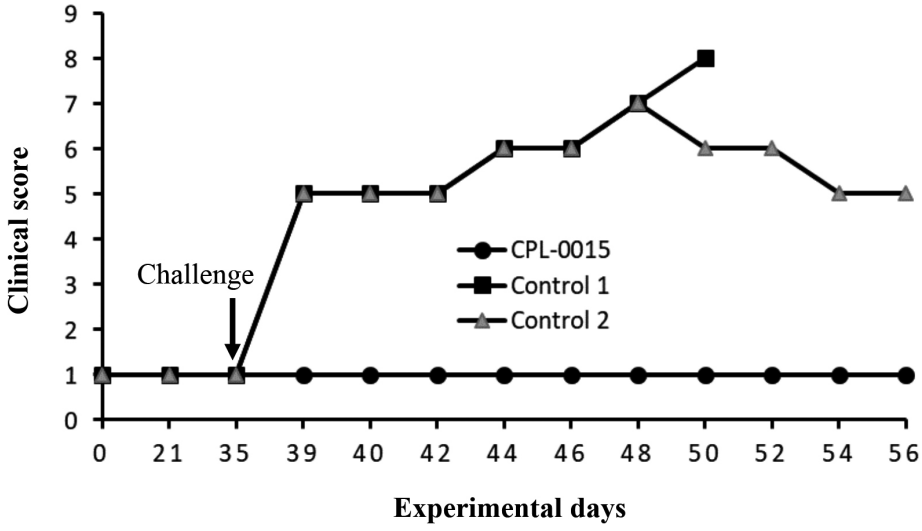

Figure 3. Clinical evaluation of Beagle dogs immunised with strain CPL-0015 (and of negative controls) challenged with the virulent Cornell-780916 strain.

Note: Group vaccinated with CPL 0015 is composed by 6 animals and control group by 2 animals.

one of them progressed towards anorexia, weakness, and prostration, and had to be euthanised.

The remarkable biological differences in terms of seroconversion and survival without clinical signs of disease after the challenge observed between inoculated and control animals demonstrate the ability of the Cuban isolate to induce a powerful protective humoral response. These results are extremely important since they show that the CPL-0015 isolate retained its immunogenic capacity during the process of adaptation to cell culture and was able to induce anti-CPV antibodies in sufficient quantities to neutralise the pathogenic strain (Puentes 2012).

When comparing this result with others reported in the international scientific literature, it can be concluded that the benefits of the CPL-0015 isolate in terms of protection are similar to those obtained with the 17/80 ISS (Pratelli *et al* 2001), Cornell-780916-115 (De Cramer *et al* 2011), 154, and NL-35-D (Larson and Schultz 2008, Siedek *et al* 2011) vaccine strains; all of which are type 2 strains, attenuated in cell culture, and inoculated using a similar route of administration. It is important to highlight that the last two abovementioned vaccine strains, despite being administered with the same biphasic scheme, required a higher viral titer ($10^{7.0}$ $TCID_{50}$/mL) than the one used in this study ($10^{6.0}$ $TCID_{50}$/mL) to achieve an effective protective status (Larson *et al* 2008, Siedek *et al* 2011).

The death of 50% of the unvaccinated animals challenged confirm the virulence of the Cornell-780916 strain. This value also corresponded with that reported in the international literature for this type of experimental group (Hernández *et al* 2015, Khatri *et al* 2017). Although there are some variations in the appearance and duration of the clinical signs of CPV, the results of this study were similar to those reported in previously published studies. The onset of disease on day 4 after the challenge is in line with the results described by other authors (Spibey *et al* 2008, Wilson *et al* 2013, Khatri *et al* 2017, Mukthar *et al* 2021) regardless of the challenge strain used, whereas the appearance of fever and mucoid or haemorrhagic diarrhea occurred at similar moments as those described historically by Meunier *et al* 1985, during experimental infections.

These results, together with the absence of the CPL-0015 strain virulence reversal (data not presented), leave open the possibility of continuing with the development of the cuban vaccine formulation. However, there is still a long way to go, the duration of immunity and stability of the formulation should be explored immediately; as well as the optimization of the vaccination scheme (biphasic and triphasic) against the interference of antiparvovirus antibodies of maternal origin, among other aspects. The perception of the international use of the future cuban vaccine requires evaluating its behaviour against pathogenic antigenic variants: CPV-2a, CPV-2b, and CPV-2c (non-homologous strains).

Based on the experimental model used and the described experimental conditions, it cis concluded that the cuban isolate, CPL-0015, proved to be safe and induced effective protection against homologous virulent strains of canine parvovirus.

REFERENCES

Altman KD, Kelman M, Ward MP. 2017. Are vaccine strain, type or administration protocol risk factors for canine parvovirus vaccine failure? *Vet Microbiol* 210, 8-16.

Aponte FA, Gómez RV, López YM. 2020. Factores predisponentes a la parvovirosis canina registrados en un hospital de Cuautitlan, México. *Rev Vet* 31, 42-45.

Cunegündes GM, Gomes AL, Viana BF, Paes LH, Shimoya Aldo, *et al*. 2008. Production and use of egg-yolk antibody for detection of canine parvovirus in feces. *Rev Cin Méd Biol* 7, 241-248.

Day M. 2008. Vaccine side effects: Fact and fiction. *Vet Microbiol* 117, 51-58.

De Cramer KG, Stylianides E, Van Vuuren M. 2011. Efficacy of vaccination at 4 and 6 weeks in the control of parvovirus canine. *Vet Microbiol* 149, 126-132.

Decaro N, Buonavoglia C, Barrs VR. 2020. Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? *Vet Microbiol* 247, 108760.

Díaz C, Correa J, Vera V. 2008. Aspectos moleculares del virus de la parvovirosis canina y sus implicaciones en la enfermedad. *Rev Med Vet* 15, 57-65.

Domínguez OA, Polanco R, Cossío G, Morejón Y, Riquenes Y. 2014. Current trends and perspectives in veterinary vaccine production. *Biotec Apl* 31, 196-203.

Domínguez OA, Acosta DP, Oliva LM, Rosales BK. 2021. Vacuna cubana contra *Pasteurella multocida* cunícula: 52 años de inmunización. *Rev Salud Anim* 43, 1-3.

Dong B, Zhang G, Zhang J, Bai J, Lin W. 2020. A systematic literature review and meta-analysis of characterization of canine parvoviruses 2 prevalent in mainland China. *Virol J* 17, 1-10.

Fresneda A, Contreras D, Paneque M, de la Cruz L, Veda C. 2015. Caracterización molecular de un aislamiento cubano de parvovirus canino. *VacciMonitor* 24, 113-119.

Hao X, He Y, Wang C, Xiao W, Liu R, *et al*. 2020. The increasing prevalence of CPV-2c in domestic dogs in China. *Peer J* 8, e9869.

Hernández BB, Catala LF. 2015. Are licensed canine parvovirus (CPV2 and CPV2b) vaccines able to elicit protection against CPV2c subtype in puppies?: a systematic review of controlled clinical trials. *Vet Microbiol* 180, 1-9.

Khatri R, Poonam MH, Minakshi CS. 2017. Epidemiology, pathogenesis, diagnosis and treatment of canine parvovirus disease in dogs: A mini review abstract. *J Vet Sci Med Diagn* 6, 2.

Langeveld PJ, Brennan RF, Martínez TJ, Jones DT, Boshuizen SR, *et al*. 2001. Inactivated recombinant plant virus protects dogs from a lethal challenge with canine parvovirus *Vaccine* 19, 3661-3670.

Larson LJ, Schultz RD. 2008. Do two current canine parvovirus type 2 and 2b vaccines provide protection against the new type 2c variant. *Vet Ther* 9, 94-101.

Li G, Ji S, Zhai X, Zhang Y, Liu J, *et al*. 2017. Evolutionary and genetic analysis of the VP2 gene of canine parvovirus. *BMC Genomics* 18, 1-13.

Lugo S, Espinosa C, Zamora B, Riera O, Sosa T, *et al*. 2019. Caracterización microbiológica y genotípica de cepas de *Pasteurella multocida* asociadas al síndrome respiratorio cunícula. *Rev Salud Anim* 41, 1-11.

Meunier PC, Cooper BJ, Appel MJ, Lanieu ME, Slauson DO. 1985. Pathogenesis of canine parvovirus enteritis: sequential virus distribution and passive inmunization studies. *Vet Pathol* 22, 617-624.

Miranda C, Thompson G. 2016. Canine parvovirus: the worldwide occurrence of antigenic variants. *J Gen Virol* 97, 2043-2057.

Moore G, Hogen E. 2010. Adverse vaccinal events in dogs and cats. *Vet Clin Smal Anim* 40, 393-407.

Mukthar M, Hasan M. 2021. Update on canine parvovirus infection: a review from the literature. *Vet Sc Res Rev* 7, 92-100.

Oliveira HS, Geraldes PF, Lopes RJ, D'Ávila VG, Fischer G, *et al*. 2010. Exposure of pampas fox (*Pseudalopex gymnocercus*) and crab-eating fox (*Cerdocyon thous*) from the Southern region of Brazil to canine distemper virus (CDV), canine parvovirus (CPV) and canine coronavirus (CCoV). *Braz Arch Biol Technol* 53, 593-597.

Peña GI, Vidal FF, Hernández RA. 2020. Factores de riesgo de la parvovirosis canina en cuatro localidades de Cuba. *Rev Inv Vet Perú* 31, e17824.

Pino RD, Márquez AM, Torres GC, Matos RR, Zamora MY. 2019. Factores de riesgo asociados a la seroprevalencia de parvovirus canino en perros del municipio Boyeros, La Habana, Cuba. *Rev Salud Anim* 41, 1-6.

Pratelli A, Cavalli A, Martella V, Tempesta M, Decaro N, *et al*. 2001. Canine parvovirus vaccination: comparison of neutralising antibody responses in pups after inoculation with CPV2 or CPV2b modified live virus vaccines. *Clin Diag Lab Immunol* 8, 612-615.

Puentes PR. 2012. Parvovirosis canina: situación actual y protección de las vacunas contra las nuevas variantes virales circulantes en la región. *Vet* 48, 5-10.

Puentes RR, Eliopulos N, Pérez R, Franco G, Sosa K, *et al*. 2012. Isolation and characterization of canine parvovirus type 2c (CPV-2c) from symptomatic puppies. *Braz J Microb* 1005-1009.

Qi S, Zhao J, Guo D, Sun D. 2020. A Mini-Review on the epidemiology of canine parvovirus in China. *Front Vet Sci* 7, 5-11.

Sebastian BP, Felipetto CJ, Kenji ME,Weiblen R, Furtado FE. 2019. New variants of canine parvovirus in dogs in southern Brazil. *Arch Virol* 164, 1361-1369.

Siedek EM, Schmidt H, Sture GH, Raue R. 2011. Vaccination with canine parvovirus type 2 (CPV-2) protects against challenge with virulent CPV-2b and CPV-2c. *Berl Munch Tierarztl Wochenschr* 124, 58-64.

Spibey N, Greenwood N, Sutton D, Chalmers W, Tarpey I. 2008. Canine parvovirus type 2 protects against virulent challenge with type 2c virus. *Vet Microbiol* 128, 48-25.

Voorhees IE, Lee H, Allison AB, Lopez-Astacio R, Goodman LB, *et al*. 2019. Limited intrahost diversity and background evolution accompany 40 years of canine parvovirus host adaptation and spread. *J Virol* 94, e01162-19.

Wilson S, Stirling C, Borowski S, Thomas A, King V, *et al*. 2013. Vaccination of dogs with Duramune DAPPi+LC protects against pathogenic canine parvovirus type 2c challenge. *Vet Rec* 172, 662-672.

Ying EY, Peaston A, Woolford L, Khuu SJ, Wallace G, *et al*. 2020. Diagnostic challenges in canine parvovirus 2c in vaccine failure cases. *Viruses* 12, 980-93.

Zhou P, Zeng W, Zhang X, Li S. 2017. The genetic evolution of canine parvovirus-A new perspective. *PLoS ONE* 12, e0175035.

Zhuang QY, Qiu Y, Pan ZH. 2019. Genome sequence characterization of canine parvoviruses prevalent in the Sichuan province of China. *Transbound Emerg Dis* 66, 897-907.

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SHORT COMMUNICATION

Fascioliasis prevalence in livestock from abattoirs in southern Chile

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ABSTRACT. Fascioliasis is a widely distributed parasitic zoonosis caused by the trematode *Fasciola hepatica* that affects livestock production and generates high economic losses. In Chilean authorised abattoirs, the infected livestock livers are condemned during the veterinary inspection. This study aims to evaluate the prevalence of fascioliasis in Chile from 2014 to 2016 and also monthly from 2002 to 2015 in livestock (cattle, pig, sheep, horse and goat) slaughtered in abattoirs of La Araucanía region, southern Chile. To do this, the available records on abattoirs provided by the sanitary authority were analysed. A descriptive statistics and trend analysis of the data by jointpoint regression was carried out. The Biobío and La Araucanía regions registered the highest levels of parasitosis in the country recording levels of 59.18 and 44.74%, respectively, and presented 50.03% of the liver condemnation rate in cattle. During the study period, a total of 2,239,164 animals were slaughtered and 40.59% infected livers with *F. hepatica* were condemned in the 9 existing abattoirs of La Araucanía region. The abattoirs located in the cities of Temuco (51.43%) and Angol (65.09%) recorded the highest percentages of fascioliasis. The cattle species recorded the highest number of slaughtered animals and liver condemnation (54.52%). The presence of the parasite was recorded annually and monthly and it was possible to observe a slight increase in fascioliasis over the years. This study provides updated information on the fascioliasis prevalence in the country and the dynamics of condemnation in endemic areas such as La Araucanía region, which could contribute to the control and prevention of this zoonosis.

Key words: abattoir, Chile, fascioliasis, livestock.

INTRODUCTION

Fascioliasis is a zoonotic parasitic disease produced by *Fasciola hepatica*, a trematode of the Phylum Platyhelminthes globally distributed and found in every continent except Antarctica (Rojo-Vazquez and Ferre 1999). It seriously affects livestock, particularly sheep and cattle, impacting public health and causing significant productive and economic losses derived from the clinical picture and the condemnation of the liver (Mas-Coma *et al* 2009). The infection is caused by the intake of short-stemmed vegetation and waters contaminated with metacercariae of the parasite (Mas-Coma *et al* 2014). The World Health Organization defines human fascioliasis as a neglected reemerging tropical disease, with endemic and epidemic outbreaks throughout the world (WHO 2013). In cattle, the effects of this pathology in chronic stages are correlated with productivity, generating weight loss, reduced milk production and lower fleece quality (Schweizer *et al* 2005).

In Chile, the disease is mainly associated with cattle and sheep. The most frequent production system involving these species is extensive grazing, which exposes the animal to various parasitic pathogens such as gastrointestinal nematodes and *F. hepatica* (Peña-Espinoza 2018).

The Chilean Agriculture and Livestock Service (SAG) regulates the slaughter of animals for consumption in authorised abattoirs. Through breeding protocols, prior examination and post-slaughter of productive species, SAG ensures the safety of slaughter livestock and their derivatives[1], guaranteeing the organoleptic quality and preventing risks for the consumer. These practices are regulated by Law N° 19.162 of the Ministry of Agriculture[2] and General Technical Standard N° 62 of the Ministry of Health[1]. During the veterinary inspection, transverse incisions are made into the liver to search for adults of *F. hepatica* or pathological lesions in the bile ducts. After detection, the condemned livers are classified as unfit for human consumption, generating a record that is kept by each abattoir[1].

Currently, there is little updated research on the prevalence of *F. hepatica* or fascioliasis that provides information on the situation at both national and regional levels. This study aims to analyse the prevalence of the

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[1] Ministerio de Salud. 2009. Norma General Técnica N° 62 Sobre inspección Medico-Veterinaria de reses y sus carnes. *Biblioteca Del Congreso Nacional*. Available at: https://www.leychile.cl/N?i=1064158&f=2009-09-14&p=

[2] Ministerio de Agricultura. 1992. Ley 19.162, Sistema obligatorio de clasificación de ganado, tipificación y nomenclatura de sus carnes y regula funcionamiento de mataderos, frigoríficos y establecimientos de la industria de la carne. *Biblioteca Del Congreso Nacional*. Available at: https://www.leychile.cl/N?i=30529&f=2009-07-03&p=

parasitosis through liver condemnation of infected livestock due to fascioliasis using the available SAG databases from authorised abattoirs, with information corresponding to Chile from 2014 to 2016 and the endemic region of La Araucanía during the period 2002-2015.

MATERIAL AND METHODS

The "Report on the Slaughtered Animals and Pathological Findings in National Abattoirs" was accessed, which contains the records of the main pathologies of interest to public health detected in slaughter animals from authorised abattoirs in Chile from 2014 to 2016. The Ñuble region was included in the statistics of the Biobío region since the law for the creation of the first was promulgated in August 2017[3]. Also, the "Statistics on SAG Abattoir Inspection" of La Araucanía region were accessed for the period 2002-2015.

The national registry is organised in two main sections: slaughtered animals (or animal gain) and pathological findings in abattoirs by year. The national and regional registry included the total number of animals by species and findings according to pathologies (echinococcosis, tuberculosis, fascioliasis, cysticercosis and trichinosis). The data corresponding to La Araucanía region was registered by month and year. The data did not include livestock traceability and the recordings are made at the end of the animal's life.

We recorded the number of both slaughtered animals and liver condemnations appearing in the national registry by region (Arica y Parinacota, Tarapacá, Coquimbo, Valparaíso, Metropolitana, O'Higgins, Maule, Biobío, La Araucanía, Los Ríos, Los Lagos, Aysén and Magallanes regions) and productive species (cattle, pig, sheep, horse and goat). The data of La Araucanía was organised by year, month and nine authorised abattoirs, with the productive livestock being grouped into the five categories previously mentioned. La Araucanía region is one of Chile's 16 administrative divisions, located at the coordinates 38°54'S 72°40'W. The studied abattoirs were Frigorífico Temuco S.A. (Temuco), Faenadora de Carnes Angol (Angol), Faenadora de Carnes Victoria S.A. (Victoria), Comercial Frigosur Rio Toltén Ltda. (Pitrufquén), Sociedad Faenadora y Comercializadora de Productos Alimenticios Araneda Ltda. (Imperial), Sociedad Matadero Chol Chol Ltda. (Chol Chol) and Matadero Industrial Lautaro (Lautaro). Also included were the abattoirs located in Galvarino and Hualpín which ceased functions in October 2005 and December 2006, respectively.

STATISTICAL ANALYSIS

The database was elaborated using a Microsoft Excel worksheet and exported to STATA15 software (StataCorp LLC, College Station, Texas, U.S.A.) for statistical analysis. The basic statistical analysis included the estimation of prevalence and mean of the prevalence over time. The trend analysis of the prevalence through time was done using a Joinpoint Regression (Kim *et al* 2000) and the Trend Analysis Software of the NIH[4]. The graphs were obtained using the GraphPad Prism version 7.00 software (GraphPad Software, La Jolla, California U.S.A.).

RESULTS AND DISCUSSION

The annual record of the statistics and trends of the most commonly detected pathologies in abattoirs at national level allowed us to perform passive monitoring to support the sanitary and zoonotic management in the country. In all the regions with authorised abattoirs of the country, *F. hepatica* was found in livers of productive animals in authorised abattoirs during the years 2014-2016.

In the early 1990s, the distribution and trend of fascioliasis in Chilean abattoirs was 30.1% for cattle (Morales and Luengo 2000). There is a great variation in the prevalence recorded in Latin America where countries such as Brazil, with industrial livestock, report numbers of 18.6% for the state of Rio Grande do Sul and 24.9% for the state of Espíritu Santo, with 7.32% being recorded between 2002 and 2011 nationwide (Molento *et al* 2018). In Uruguay, parasitosis exceeds 50% prevalence in productive farms (Sanchís *et al* 2015) while in Mexico, where human fascioliasis is significant (Mas-Coma *et al* 2014), condemnation in abattoirs due to *F. hepatica* reaches 20.99% (Rangel and Martínez 1994). On the other hand, Costa Rica has low prevalence levels (1.82%) of adults of *F. hepatica* after veterinary inspection (Rojas and Cartín 2016). Likewise, in the state of Lara, Venezuela, 8.49% of the animals slaughtered in abattoirs were reported as fascioliasis findings (Quijada *et al* 2005). Despite the differences existing within the Latin American meat industry, during the last decade, *F. hepatica* consistently appeared in those countries.

There are few reports of the prevalence of the parasite in Chile, including human fascioliasis case reports (López *et al* 2004, Morales *et al* 2009, Rosas *et al* 2008, Venturelli *et al* 2003) and a case report on resistance to triclabendazole treatment in the Metropolitana region (Gil *et al* 2014). Fascioliasis, as a problem of veterinary concern, is mainly absent from the epidemiological and public health context, with only one report on the prevalence in endemic areas during the late 1980s (Apt *et al* 1993). The livestock is

[3] Ministerio del Interior y Seguridad Pública. 2017. Ley 21.033, Crea la XVI región de Ñuble y las provincias de Diguillín, Punilla e Itata. *Biblioteca Del Congreso Nacional*. https://www.leychile.cl/N?i=1107597&f=2018-09-06&p=

[4] National Institutes of Health. Division of Cancer Control & Population Sciences. USA. 2000. *Joinpoint Trend Analysis Software*. Available at: https://surveillance.cancer.gov/joinpoint/

mainly concentrated in the centre and south of the country where the main productive activity is located. It has been given special attention as an endemic area for the parasite (Apt *et al* 1993).

The prevalences (SD) obtained in our study raise the alert for the significant presence of the parasite in the country. The abattoirs located in Biobío 59.18 (4.32) % and La Araucanía regions 44.74 (2.87) %, (figure 1) stand out due to a prevalence similar to that reported in Zambia (64.4%) where the animals exhibit poor body condition (Nyirenda *et al* 2019). The percentage of condemned livers reaches the highest levels in endemic areas of human fascioliasis, particularly in the department of Apurimac (80.1%) in Perú (Espinoza *et al* 2010). Also, abattoirs in other regions of Chile reported a significant presence of the parasite with Los Ríos and Los Lagos regions presenting condemnations of 26.27 (1.39) % and 26.11 (2.57) %, respectively (figure 1).

Our results show a higher prevalence in the southern regions of the country, particularly in the Biobío, La Araucanía, Los Ríos and Los Lagos regions. The elevated livestock population of the area is an important factor that influences the prevalence. Moreover, it is well known that climate conditions and climate changes can affect the parasitism of the species (Hughes 2000). In the case of *F. hepatica*, this impact is conditioned by the freshwater snail, an intermediate host that determines the possibility of completing the life cycle of this zoonotic disease (Reinhard 1957) due to its sensitivity to temperature and soil moisture (Yigezu *et al* 2018). The weather in southern Chile is temperate humid, with rainfall throughout the year. The feeding systems used in the southern regions are predominantly extensive and mixed, where the grazing behaviour of the livestock allows the exposure to the infective stages of *F. hepatica* (Fox *et al* 2013), unlike the case of more intensive systems used in Metropolitana 24.91 (4.10) %, O'Higgins 0.22 (0.02) % and Maule 6.80 (0.56) % regions. The conditions related to the prevalence are shown in figure 1.

The collected data was obtained from the authorised abattoirs at the end of the productive life of the animals. Therefore, the analysis does not consider livestock traceability making impossible to determine where the animals getting infected are or their movement between regions. Further studies that include traceability are needed to enrich the investigation.

The national record displays a total of 20,005,649 animals slaughtered during the study period, with the liver condemnation rate (SD) in cattle being 50.03 (1.56) % nationwide (figure 2). Previous reports in the early 1990s showed a lower prevalence of 30.1% for the species (Morales and Luengo 2000). The second most infected productive species was equine with 18.3 (1.59) % where the parasitosis was similar to previous reports on fascioliasis in equines. Morales *et al* (2009) reported 12.3%, a similar study found 13.54% (Apt *et al* 1993) and a recent work described 10.4% in racehorses (Muñoz *et al* 2008). In the other species, our results showed that goats reach 1.29 (1.35) % as opposed to the findings of Morales and Luengo (2000) with 14%. The least parasitised livestock species were ovines and porcines with 0.28 (0.05) % and 0.01 (0.01) % respectively, corresponding with a downward trend of this species as reported in previous works (Morales and Luengo 2000), but in disagreement with the 20.61% reported by Apt *et al* (1993). In cattle and equine species, the prevalence observed is related to previous reports but an increase in prevalence is observed.

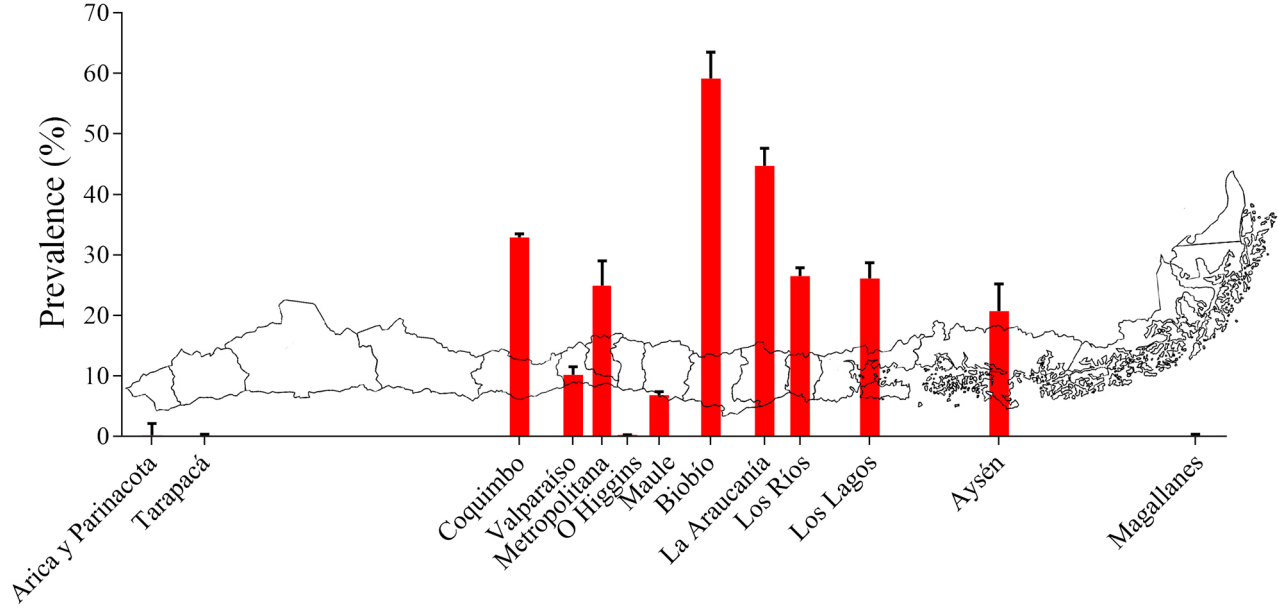

Figure 1. Prevalence of fascioliasis (% red) in Chile abattoirs, during the period 2014-2016. Standard deviation (T) is displayed.

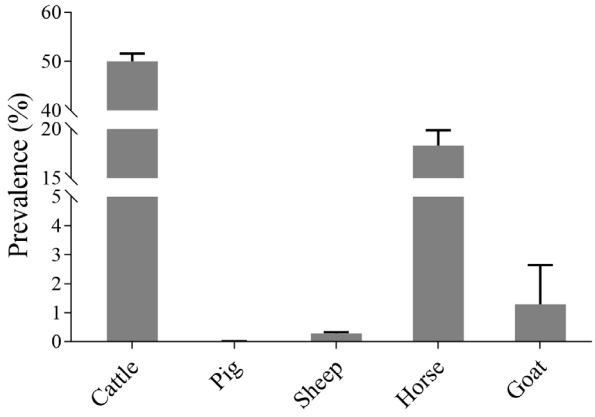

Figure 2. Prevalence of fascioliasis in main productive species slaughtered in Chile abattoirs from 2014 to 2016. Standard deviation (T) is displayed.

Between 2002 and 2015, a total of 2,239,164 animals were slaughtered in La Araucanía region, reporting condemnations of 40.59% due to *F. hepatica* (908,901 animals with findings) (see supplementary material 1). The highest levels of fascioliasis correspond to the abattoirs located in Angol and Temuco (65.09 and 51.43%, respectively) (figure 3). Microclimates are crucial for the distribution of vectors, this particularly concerns diseases transmitted by water-borne vectors such as freshwater snails (Mas-Coma *et al* 1999). The increase in winter temperatures raise the metabolic rate, egg production and feeding frequency of invertebrates (De La Rocque *et al* 2008). The Angol borough has mediterranean climate with mild temperatures and rainy winters and its weather is warmer than in other boroughs of the central valley in the same region, where the abattoirs of Temuco, Victoria (32.49%), Lautaro (3.33%) and Pitrufquén (9.51%) are located (figure 3). This microclimate would explain the prevalence exhibited by the Angol abattoir, considering

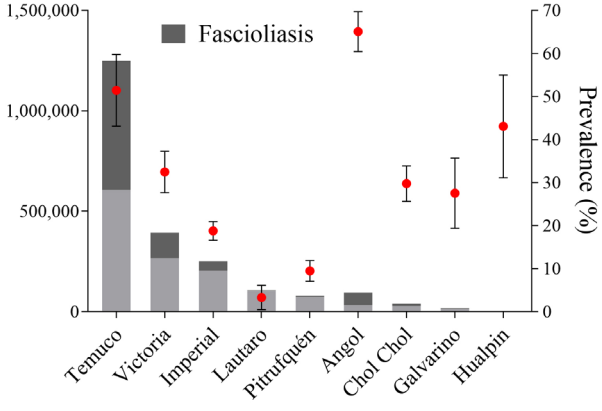

Figure 3. Number of slaughtered animals (columns) and fascioliasis (dark grey), prevalence of fascioliasis (%) (red) and 95% confidence interval (T) by abattoir in La Araucanía region, during the period 2002-2015.

that it recorded only 95,571 slaughtered animals during the studied period.

The rural boroughs of Galvarino and Hualpín range within the lowest quintile of the percentage of the population living with poverty income (Ministerio de Desarrollo Social 2015) and in less than 5 years of operation it registered a high level of condemnation due to *F. hepatica*, recording 27.56 and 43.09%, respectively (figure 3). The uptime of abattoirs and missing data were recorded (see supplementary material 2). La Araucanía is the region with the lowest economic income up to 2017 (Ministerio de Desarrollo Social 2017) with a rural population of 29.1% which is higher than the national average (INE 2017). These factors contribute negatively to control this or any parasitosis, harming livestock and health management. The use of an extensive system and veranadas[5] conditions animal movement to agroecological areas with greater vegetable mass and annual precipitation (Catrileo 2015), propitiating the distribution and seasonality of the intermediary host (Mas-Coma *et al* 2009). Those conditions allow permanent infection in the areas used for grazing, being associated with the prevalence exhibited.

The dynamic behaviour shows an increase in the number of cattle associated with the increasing demand for the product in the national reports (Cofré 2019, INE 2019), with a marked difference between 2007 (138,978 total animals) and 2008 (191,072 total animals). Our results exhibit three periods, only the first period between 2002 and 2007, characterised by high prevalence indices, was statistically significant (P=0.0048) with an annual percent change of 21.7, reaching 60.09% in 2007, the highest record of condemnations for the period studied. The two periods between 2008 and 2015 were not significant, recording 45.19% in condemnations in 2015 (figure 4). This behaviour is probably due to the modification made to the regulation on the veterinary inspection of cattle and their meats, General Technical Standard N°62[1], which was promulgated in July 2002 and entered into force in 2009. The main modification was the certification by SAG-accredited professionals of the control and monitoring of cattle and their derivatives, reforming the requirements of the abattoirs. Therefore, the impact on livestock production and the control of infectious diseases by producers and government services resulted in the improvement of sanitary programs for cattle.

According to our results, slaughter increased the most in December (225,710 total of animals) which is linked to an increase in consumption due to the end of the year celebrations (Cofré 2019). Studies have suggested that the prevalence of *F. hepatica* is conditioned by the ecology of its intermediary host and aquatic vector, a lymnaeid of the genus *Galba* (Artigas *et al* 2011). Considering its habitat

[5] Yáñez Barrios L. 2018. Región de la Araucanía, Información regional. *Oficina de Estudios y Políticas Agrarias*, 16. Available at: www.odepa.gob.cl

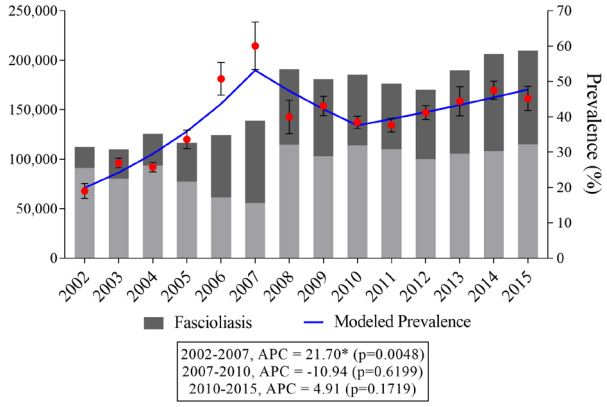

Figure 4. Number of slaughtered animals (columns) and fascioliasis (dark grey), prevalence of fascioliasis (%) (red), 95% confidence interval over time (T) and modeled prevalence (blue), during the period 2002-2015 in La Araucanía region. *Indicates that the Annual Percent Change (APC) is significantly greater than zero (Jointpoint regression).

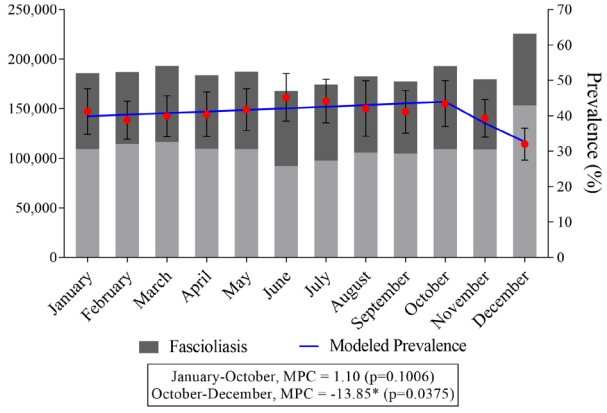

Figure 5. Number of slaughtered animals (columns) and fascioliasis (dark grey), prevalence of fascioliasis (%) (red), 95% confidence interval over time (T) and modeled prevalence (blue), by month in La Araucanía region, during the period 2002-2015. *Indicates that the Monthly Percent Change (MPC) is significantly smaller than zero (Jointpoint regression).

and the climatic conditions of the area, the increase in precipitation and temperature would in turn increase the snail populations (Müller *et al* 1999). Our results show that during the rainy season in the Southern Hemisphere, June (45.20%), July (44.15%) and August (42.09%), there was a lower intake number and high numbers of condemnations (figure 5). This is related with prevalence reports during ideal seasons for their development, in countries such as Venezuela (Quijada *et al* 2005), China (Yuan *et al* 2016), Vietnam (Nguyen and Nguyen 2012) and Cuba (Palacio Collado *et al* 2017), suggesting an even higher probability of becoming infected in the rainy season (Byrne *et al* 2018, Novobilský *et al* 2014). However, the change in monthly percentage through this period was not significant. On the other hand, the prevalence between the spring months - November and December - shows a statistically significant (*P*=0.0375) lower index (39.32 and 32.00 %, each) (figure 5), probably associated with preventive deworming which takes place at the beginning of the spring as part of government programs. It is important to emphasise the permanent indices throughout the year. The half-life of *F. hepatica* has been reported to be more than two years in livestock (Mas-Coma *et al* 2014); the constant elimination of eggs from the definitive host (Robles-Pérez *et al* 2015) and the invariable presence of the intermediary host in the area (Mas-Coma *et al* 1999) causes constant reinfection.

The most relevant among the productive species was cattle which registered findings of fascioliasis in the abattoirs of Temuco (51.43%) and Victoria (32.49%). The importance of cattle species and the parasitic prevalence of *F. hepatica* had been previously reported at national level (Morales and Luengo 2000). Smaller productive species such as pigs, sheep and goats are traditionally home slaughtered for private consumption although it is an illegal activity without health certification[2] that is not included in official abattoir records.

The prevalence observed is related to the definitive host tolerance to the infection (Rojo-Vazquez and Ferre 1999). The greatest percentage was reported in cattle (54.52%) because it exhibited low receptivity and delay in response to the implantation process in the liver (Boray 1999, Boray *et al* 1969). In more receptive mammals, such as goat (4.84%) and sheep (4.68%), there is a high parasitic activity and noticeable pathogenicity, producing acute symptoms that in most cases are lethal (Boray 1999, Boray 1985). On the other hand, we recorded low prevalence in pig (0.39%) and horse (3.65%) which confirms that they respond more quickly to hepatic invasion (figure 6) (Boray *et al* 1969).

In conclusion, fascioliasis is the main cause of condemnation in slaughtered livestock at national and regional levels, with cattle reporting the highest prevalence.

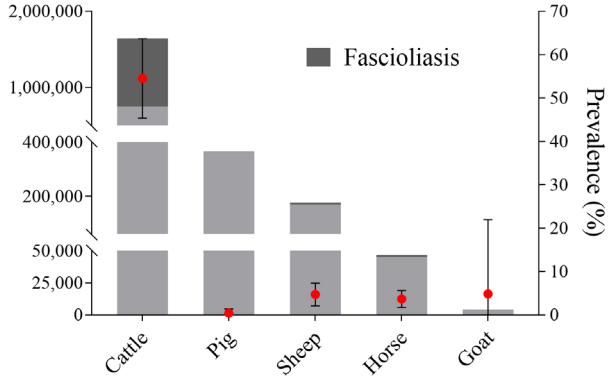

Figure 6. Number of slaughtered animals (columns) and fascioliasis (dark grey), prevalence of fascioliasis (%) (red) and 95% confidence interval (T) by main productive species in La Araucanía region, during the period 2002-2015.

The weather as well the productive system are associated with the prevalence found between the centre and the south regions of the country. The Biobío and La Araucanía regions show the highest rates in Chile and, in particular, La Araucanía region presents a high prevalence of *F. hepatica*. The presence of the intermediary host favoured by the climatic conditions of the area ensures the cycle of the parasite, reporting constant prevalence with a low variation during the chronological year. The dynamic behaviour of the parasitosis reports a slight increase in the prevalence through the years studied. Our results indicate a constant and evident prevalence of the parasite to the detriment of the productive economy and the organoleptic quality of the product due to the inflammatory process. The analysis of condemnation records at national and regional levels is a representative methodology to determine prevalence and monitoring of the disease and is useful for the epidemiological control and management of *F. hepatica*.

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REFERENCES

Apt W, Aguilera X, Vega F, Alcaíno H, Zulantay I, *et al*. 1993. Prevalencia de fascioliasis en humanos, caballos, cerdos y conejos silvestres, en tres provincias de Chile. *Boletin de La Oficina Sanitaria Panamericana* 115, 405-414.

Artigas P, Bargues MD, Mera RL, Agramunt VH. 2011. Characterisation of fascioliasis lymnaeid intermediate hosts from Chile by DNA sequencing, with emphasis on *Lymnaea viator* and *Galba truncatula*. *Acta Tropica* 120, 245-257.

Boray J. 1985. Fluke of domestic animals. In: Graafat SM, Howard WE, Marsh RE (eds). *Parasites and Predators*. Elsevier; New York, USA, Pp 179-185.

Boray J. 1999. Liver fluke disease in sheep and cattle. *AGFACTS NSW Agriculture* 1-16.

Boray J, Happich FA, Andrews JC. 1969. The epidemiology of Fasciolosis in two representative endemic regions of Australia. *Aust Vet J* 45, 549-553.

Byrne AW, Graham J, McConville J, Milne G, McDowell S, *et al*. 2018. Seasonal variation of Fasciola hepatica antibodies in dairy herds in Northern Ireland measured by bulk tank milk ELISA. *Parasitol Res* 117, 2725-2733.

Catrileo A. 2015. Decisiones de manejo en producción de carne bovina. *Boletín INIA* 316. Temuco, Chile.

Cofré CR. 2019. *Comportamiento y caracterización de la producción de ganado bovino en Chile*. INE, Santiago, Chile.

De La Rocque S, Rioux JA, Slingenbergh J. 2008. Climate change: effects on animal disease systems and implications for surveillance and control. *Rev Sci Tech* 27 339-354.

Espinoza JR, Terashima A, Herrera-Velit P, Marcos LA. 2010. Fasciolosis humana y animal en el Perú: Impacto en la economía de las zonas endémicas. *Revista Peruana de Medicina Experimental y Salud Pública* 4, 604-612.

Fox NJ, Marion G, Davidson RS, White PCL, Hutchings MR. 2013. Modelling parasite transmission in a grazing system: The importance of host behaviour and immunity. *PLoS ONE* 8.

Gil LC, Díaz A, Rueda C, Martínez C, Castillo D, *et al*. 2014. Fascioliasis hepática humana: Resistencia al tratamiento con triclabendazol. *Revista Med Chile* 142, 1330-1333.

Hughes L. 2000. Biological consequences of global warming: is the signal already apparent? *Trends Ecol Evol* 15, 56-61.

INE, Instituto Nacional de Estadísticas. 2017. Memoria CENSO 2017. *Instituto Nacional de Estadística*, Santiago, Chile.

INE, Instituto Nacional de Estadísticas. 2019. Ferias y mataderos, trimestre enero-marzo 2019. *Instituto Nacional de Estadística* 6, 6-8.

Kim H, Fay MP, Feuer E J, Midthune DN. 2000. *Permutation test for joinpoint regression with applications to cancer rates*. 351, 335-351.

López A, Silva C, Busel D. 2004. Fasciolasis hepática: Reporte de un caso y revisión de la literatura. *Revista Chilena de Radiología* 10, 118-123.

Mas-Coma S, Anglés R, Esteban JG, Bargues MD, Buchon P, *et al*. 1999. The Northern Bolivian Altiplano: A region highly endemic for human fascioliasis. *Trop Med Int Health* 4, 454-467.

Mas-Coma S, Valero MA, Bargues MD. 2009. Climate change effects on trematodiases , with emphasis on zoonotic fascioliasis and schistosomiasis. *Vet Parasitol* 163, 264-280.

Mas-Coma S, Valero MA, Bargues MD. 2009. Chapter 2 *Fasciola*, Lymnaeids and Human Fascioliasis, with a Global Overview on Disease Transmission, Epidemiology, Evolutionary Genetics, Molecular Epidemiology and Control. *Adv Parasit* 69, 41-146.

Mas-Coma S, Valero MA, Bargues MD. 2014. Fascioliasis. In: Toledo R, Fried B (eds). *Digenetic Trematodes*. Springer, New Yor, USA, Pp 77-114.

Ministerio de Desarrollo Social. 2015. *Informe de Desarrollo Social CASEN, La Araucanía. Encuesta de Caracterización Socioeconómica Nacional*. Ministerio de Desarrollo Social, Santiago, Chile.

Ministerio de Desarrollo Social. 2017. *Informe de Desarrollo Social CASEN Nacional. Encuesta de Caracterización Socioeconómica Nacional*. Ministerio de Desarrollo Social, Santiago, Chile.

Molento MB, Bennema S, Bertot J, Pritsch IC. 2018. Bovine fascioliasis in Brazil : Economic impact and forecasting. *Veterinary Parasitology: Regional Studies and Reports* 12, 1-3.

Morales MA, Luengo J. 2000. Distribución y tendencia de la fasciolosis en ganado de abasto en Chile. *Parasitología Al Día* 24, 9-11.

Morales GJL, Arriagada HR, Salas GL, Morales AC, Fuentes AF, *et al*. 2009. Hematoma subcapsular por Fasciolasis. *Revista Chilena de Cirugía* 61, 73-77.

Müller G, Berne M, Raffi L, Jesus L, Paulsen R, *et al.* 1999. Influences of temperature on longevity of metacercarie of *Fasciola hepatica*. *Revista Brasileira de Agrociencia* 5, 164-165.

Muñoz L, Rubilar L, Zamora D, Sepúlveda O, Rehhof C, *et al*. 2008. Fasciolosis en equinos fina sangre de carrera del Club Hípico Concepción, Chile. *Parasitología Latinoamericana* 63, 88-91.

Nguyen ST, Nguyen DT. 2012. Prevalence of *Fasciola* in cattle and of its intermediate host Lymnaea snails in central Vietnam. *Trop Anim Health Pro*, 1847-1853.

Novobilský A, Engström A, Sollenberg S, Gustafsson K, Morrison DA, *et al*. 2014. Transmission patterns of *Fasciola hepatica* to ruminants in sweden. *Vet Parasitol* 203, 276-286.

Nyirenda SS, Sakala M, Moonde L, Kayesa E, Fandamu P, *et al*. 2019. Prevalence of bovine fascioliasis and economic impact associated with liver condemnation in abattoirs in Mongu district of Zambia. *BMC Vet Res* 15, 1-8.

Palacio Collado D, Bertot Valdés JA, Beltrao Molento M, Vázquez Gil Á, Izquierdo Pérez N, *et al*. 2017. Comportamiento estacional de *Fasciola hepatica* en bovinos sacrificados en el matadero Chacuba, Camagüey, Cuba. *Revista de Producción Animal* 29, 30-35.

Peña-Espinoza M. 2018. Drug resistance in parasitic helminths of veterinary importance in Chile: Status review and research needs. *Austral J Vet Sci* 50, 65-76.

Quijada T, Araque C, Jimenez M, Pacheco A, Quijada J, *et al*. 2005. Prevalencia de la *Fasciola hepática* en bovinos en un matadero Industrial del estado de Lara. Venezuela. *Gaceta de Ciencias Veterinarias* 10.

Rangel JL, Martínez E. 1994. Pérdidas por decomiso de hígados y distribución geográfica de la fasciolosis bovina en el estado de Tabasco, México. *Vet Mex* 25, 327-331.

Reinhard EG. 1957. Landmarks of parasitology I. The discovery of the life cycle of the liver fluke. *Exp Parasitol* 6, 208-232.

Robles-Pérez D, Martínez-Pérez JM, Rojo-Vázquez FA. 2015. Screening anthelmintic resistance to triclabendazole in *Fasciola hepatica* isolated from sheep by means of an egg hatch assay. *BMC Vet Res* 1-5.

Rojas D, Cartín JA. 2016. Prevalencia de *Fasciola hepatica* y pérdidas económicas asociadas al decomiso de hígados en tres mataderos de clase a de Costa Rica. *Agron Costarric* 40, 53-62.

Rojo-Vazquez FA, Ferre P. 1999. Parasitosis hepáticas, Fasciolosis. In: Cordero del Campillo M, Rojo-Vazquez FA (eds). *Parasitología Veterinaria*. McGraw-Hill Interamericana, Madrid, España, Pp 260-272.

Rosas LH, Fiestas FC, Torres MR, Aita CG, Lozada MD. 2008. Hematoma subcapsular hepatico por fasciolasis. *Revista Chilena de Radiologia* 14, 20-24.

Sanchís J, Hillyer GV, Madeira De Carvalho LM, Macchi MI, *et al*. 2015. Riesgo de exposición a *Fasciola hepatica* en ganado vacuno en extensivo de Uruguay y Portugal determinado mediante ELISA y un antígeno recombinante. *Arch Med Vet* 47, 201-208.

Schweizer G, Braun U, Deplazes P, Torgerson PR. 2005. Estimating the financial losses due to bovine fasciolosis in Switzerland. *Vet Rec* 157, 188-193.

Venturelli LA, Monje KM, Assef PV, Venturelli MF. 2003. Fasciolasis hepática. *Cuadernos de Cirugía* 17, 43-46.

WHO, World Health Organization. 2013. *Sustaining the drive to overcome the global impact of neglected tropical diseases: second WHO report on neglected tropical diseases*. Geneva, Switzerland.

Yáñez Barrios L. 2018. Región de la Araucanía, Información regional. *Oficina de Estudios y Políticas Agrarias*, 16. www.odepa.gob.cl

Yigezu G, Mandefro B, Mengesha Y, Yewhalaw D, Beyene A, *et al*. 2018. Habitat suitability modelling for predicting potential habitats of freshwater snail intermediate hosts in Omo-Gibe river basin, Southwest Ethiopia. *Ecol Inform* 45, 70-80.

Yuan W, Liu J, Lu K, Li H, Duan M, *et al.* 2016. Molecular identification and seasonal infections of species of *Fasciola* in ruminants from two provinces in China. *J Helminthol* 359-363.

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CASE REPORT

Multiple cervical spondylolisthesis and thoracic vertebral malformation in an 11 month-old Chilean Caballo Raza Chilena colt

Cristóbal Dörner[a,b*], Javiera Encalada[b], Jorge Maldonado[c], Joanie Palmero[d]

ABSTRACT. Compressive myelopathy caused by developmental cervical and thoracic malformation was diagnosed in an 11-month-old Chilean Caballo Raza Chilena colt. The patient evidenced an abnormal wide-based stance, neck muscle atrophy, upside-down neck conformation and tetra ataxia. Ataxia was exacerbated when the colt was walked down and uphill, walked with elevated head, backing and turning in tight circles. The patient showed no improvement following medical therapy, therefore, the colt was euthanised due to a poor prognosis after myelogram findings. Cervical spine malalignment (spondylolisthesis) associated with multi-level compression of the spinal cord was suspected based on cervical radiographs and myelogram findings and was confirmed postmortem. Thoracic vertebral malformation retained cartilage matrix spicules and a flare of the cranial vertebral epiphysis of the first thoracic vertebrae (T1) were also diagnosed at necropsy.

Key words: horse, cervical vertebrae, stenosis, myelogram.

Cervical vertebral malformation (CVM) (also known as cervical vertebral compressive myelopathy, wobbler syndrome and cervical stenotic myelopathy) (Reed *et al* 2007, Cardona *et al* 2013, Kühnle *et al* 2018, Szklarz *et al* 2019) is a common and widely described cause of ataxia and paresis affecting many different breeds of horses (Levine *et al* 2010). Ataxia and weakness commonly seen in horses with CVM are caused by narrowing of the cervical vertebral canal and compression of the spinal cord, often combined with malalignment and malformation of the cervical vertebrae. Two broad categories of CVM resulting in spinal cord compression have been proposed; type 1 affects young horses with compression as a result of developmental abnormalities of the cervical vertebral column, and type 2 affects older horses and typically involves a degenerative process (Nout and Reed 2003, Reed *et al* 2007).

The most important factor in the diagnosis of cervical vertebral malformation in adult horses and foals is the identification of cervical vertebral canal stenosis. The diagnosis can be made with more confidence by assessing the diameter of the vertebral canal. In many cases, cervical radiographs and myelography remain the only tools available to confirm a diagnosis of CVM and to define the site of spinal cord compression. Myelography has been considered the gold standard antemortem diagnostic test, however, sagittal diameter ratio analysis from plain radiographs may be more sensitive and specific than myelography and use of intra- and intervertebral sagittal diameter ratios may have greater predictive accuracy for diagnosis of CVM (van Biervliet *et al* 2006, Reed *et al* 2007, Hahn *et al* 2008). Although CVM is known to affect many different breeds of horses (i.e. described in 1.3% of young Thoroughbreds (Oswald *et al* 2010)) it is a condition rareley diagnosed in young and adult horses Caballo Raza Chilena and accordingly, there is no reference in the equine literature describing CVM in this breed.

The etiopathogenesis of breed predilections of CVM is still unknown but is speculated to involve genetic factors and differences in both morphometry and use. The Caballo Raza Chilena breed is characterised by a muscled short neck conformation and, furthermore, their late taming results in maintenance nutrition at younger ages avoiding overfeeding when compared to other breeds (Murúa 2006). All the above might be important factors involved in the low incidence of CVM reported in this breed. This case report describes severe cervical and thoracic malformation in an 11-month-old Caballo Raza Chilena with clinical features that, to the authors' knowledge, have not been previously described for this breed.

An 11-month-old Caballo Raza Chilena was presented to the referral hospital with progressive onset of neurologic signs, including severe ataxia of all 4 limbs. At 6-months-old, the owner noticed a ewe-necked conformation showing an upside-down neck, the development of intermittent stumbling and an abnormal stance. Clinical signs persisted until the colt was referred to the veterinary hospital. No known history of trauma was reported. Vaccination and deworming status were up to date. Diet consisted of alfalfa hay and water *ad libitum* and was deemed adequate.

At initial presentation, the colt was bright, alert, and responsive with normal vital parameters and a body condition score of 4 (range 1-9) (Henneke *et al* 1983). The colt was grade 3/5 ataxic in all 4 limbs with normal mentation and behaviour (Reed *et al* 2007). Static neurological

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Table 1. Sagittal diameter ratios and myelographic measurements for vertebral sites of the colt with cervical vertebral malformation.

Localisation	Intervertebral ratio	Intravertebral ratio	Dorsal column diameter reduction	Ventral column diameter (VB)
C2-C3	88.35%	C3 55.85%	18 %	C3= 3.49mm
C3-C4	70.81%	C4 55.50%	9.4%	C4= 3.54mm
C4-C5	74.39%	C5 53.39%	93.61%	C5= 0mm
C5-C6	76.38%	C6 58.14%	89.8%	C6= 3.65mm
C6-C7*	72.67%	C7 63.18%	-	C7= 4.25mm
C7-T1*	86.40%	T1 59.74%	-	T1= 3.78mm

*Dorsal column diameter reduction and dural diameter reduction were not obtained because the dorsal contrast column was lost at C6-C7.

exam revealed abnormal (base-wide) limb posture, neck muscle atrophy (cervical serratus, rhomboid, and splenius muscles), ventral displacement of the cervical vertebrae and trachea, and low head carriage (figure 1A). No cranial nerve deficits were observed. Gait analysis showed a severe lack of coordination of motor movements in all four limbs. Ataxia was exacerbated when the colt was walked down and uphill, walked with the head elevated, backing, and turning in tight circles. The colt also showed signs of brachial plexus compression during certain manoeuvres, became stiff in the front limbs, and almost dropped. Dynamic tail pull showed a lack of resistance, and the hind limbs were weak and easily pulled off balance followed by the colt taking several strides to recover.

The colt was treated with flunixin meglumine[1] (1 mg/kg bwt i.v b.i.d.), dexamethasone[2] (0.01 mg/kg bwt i.v s.i.d.), dimethylsulfoxide 99%[3] (100 mL in 1 L Lactated Ringer's solution[4]) i.v. s.i.d., during 4 days. Despite treatment, no improvement was observed, and the colt was referred to the veterinary hospital for further diagnostics including imaging.

Complete blood count and serum biochemistry profile were unremarkable. Standing cervical radiographs showed severe malalignment (ventral subluxation) of the cervical vertebral column between C4-C7, but no narrowing of the vertebral canal was noted (figure1B, table 1). A myelogram was subsequently performed under intravenous anaesthesia in a padded recovery room as described by Grant and Paterson (2006) (figure 2). An 18G spinal needle was placed in the atlantooccipital space, 40 mL of cerebrospinal fluid (CSF) were withdrawn, and 40 mL of contrast agent (iodixanol [Visipaque 300][5] 300mg/mL) were injected intrathecally. The myelogram revealed spinal cord compression in neutral, flexed, and extended radiographs at C5 (loss of ventral dye column within the vertebral body), between C5-C6 (thin dorsal column), and between C6-C7 (loss of dorsal dye column) (table 1). Contrast material was observed until caudal T1 (figure 3). No thoracic abnormalities of bone were presumed from plain radiographs nor myelogram. Based on these findings, developmental cervical vertebral malformation/malarticulation (spondylolisthesis) was diagnosed.

The colt recovered uneventfully from the myelogram. However, due to the myelographic findings of vertebral canal stenosis, multiple areas of spinal cord compression, and associated poor prognosis, euthanasia and postmortem evaluation were elected. At necropsy, malformation of the dorsal lamina of C5 and C7 were observed causing stenosis of the vertebral canal. Additionally, thoracic vertebral malformation associated with retained cartilage matrix spicules and a flare of the cranial vertebral epiphysis of the first thoracic vertebrae (T1) were also diagnosed at necropsy. Spinal cord histopathology was not performed, and no other organs were examined postmortem.

Compressive stenotic myelopathy is the most common non-infectious cause of spinal ataxia in young and adult horses (Levine *et al* 2010, Janes *et al* 2015). The first type of CVM occurs in young horses and is essentially developmental in which malformation/malarticulation of the cervical vertebral column causes spinal cord compression (van Biervliet *et al* 2006). Compressive vertebral malformation in young horses (<3 years old) is a multi-factorial disease affected by genetic predisposition and environmental influence such as gender, nutrition, hormonal changes, exercise, trauma, and rate of growth (van Biervliet *et al* 2006, Levine *et al* 2008), with Thoroughbreds and Warmbloods being overrepresented (Levine *et al* 2010, Piercy 2011). Different types of lesions have been reported in young horses (osteochondrosis, osseous cyst-like structures, fibrous tissue replacement of trabecular bone, retained cartilage matrix spicules, and osteosclerosis) providing evidence that developmental abnormalities during cervical vertebrae growth and

[1] Febrectal™, Dragpharma, Santiago, Chile.

[2] Hasyun™, Dragpharma, Santiago, Chile.

[3] DMSO 99%, Valhoma, Tulsa, OK.

[4] Ringer-Lactato, Baxter Chile, Santiago, Chile.

[5] Visipaque™ 300, GE Healthcare Inc. Chile, Princeton, NJ.

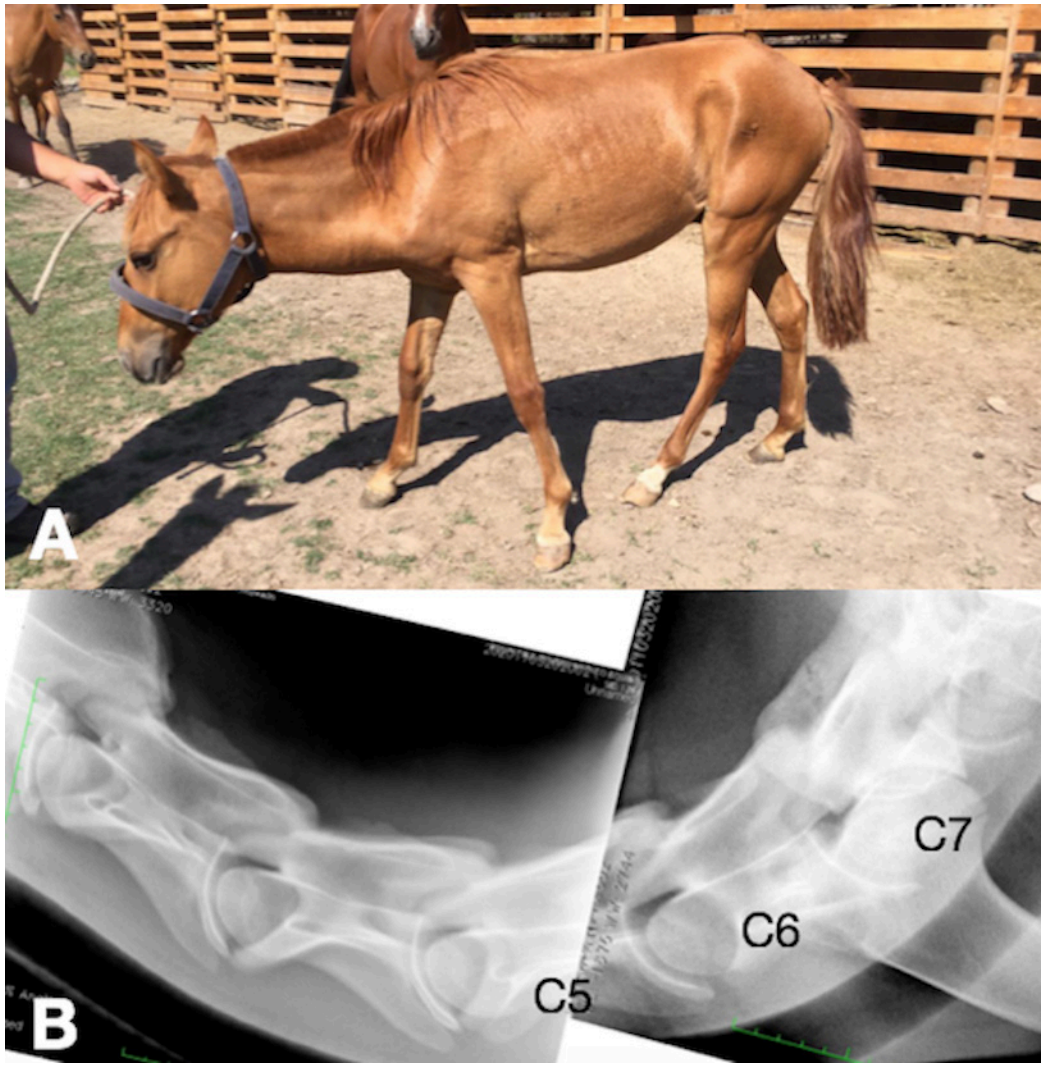

Figure 1. A) Photograph of the colt presenting a notorious alteration at mid and lower neck with low head carriage, abnormal stance in the fore and hind legs and muscle atrophy of the neck. B) Reconstruction of the colt cervical radiographs in neutral position showing malalignment (spondylolisthesis) most evident between C4-C5 and C6-C7 without apparent intervertebral space abnormalities.

maturation are important in their pathogenesis (Stewart *et al* 1991, Janes *et al* 2015, Bergmann *et al* 2020). Also, anomalous C6 with absence (symmetric or asymmetric) of the ventral lamina of the transverse process might be associated with developmental spinal stenosis (DeRouen *et al* 2016). The second type of CVM is most often seen in mature horses and has been commonly characterised by malformation with degenerative joint disease of the articular processes, wedging of the vertebral canal, periarticular proliferation with or without a synovial or epidural cyst and overt fractures of the articular processes (Reed *et al* 2007). Spinal cord compression may also result from traumatic injury (Matthews and Nout 2004, Denoix 2005), vertebral body fracture (Matthews and Nout 2004), vertebral neoplasia (Hirsch *et al* 2009), discospondylitis (Furr *et al* 1991, Denoix 2005), intervertebral disk protrusion (Nixon *et al* 1984), epidural hematoma (Cunha dos Santos *et al* 2014, MacMillan *et al* 2020), ischemic fibrocartilaginous embolism (Sebastian and Giles 2004, Dörner *et al* 2015) and arachnoid diverticulum (Allison and Moeller 2000). Furthermore, spinal cord compression may be also associated to congenital malformations such as hemivertebrae (Wong *et al* 2005), butterfly vertebrae (Rendle *et al* 2008), block vertebrae (Perris *et al* 1994), occipitoatlantoaxial malformation (Mayhew *et al* 1978, Watson and Mayhew 1986), atlantoaxial subluxation (Witte *et al* 2005) and atlantoaxial instability (Rush 2012, Cole *et al* 2017). However, congenital anomalies of the vertebral column are reported infrequently. In Chile, as elsewhere, CVM is a disease commonly diagnosed in young and adult Thoroughbreds and Warmbloods. Despite being the most prevalent cause of spinal ataxia in the abovementioned breeds, CVM is rarely noted in young and adult Chilean horses and thus has not been described in the literature in

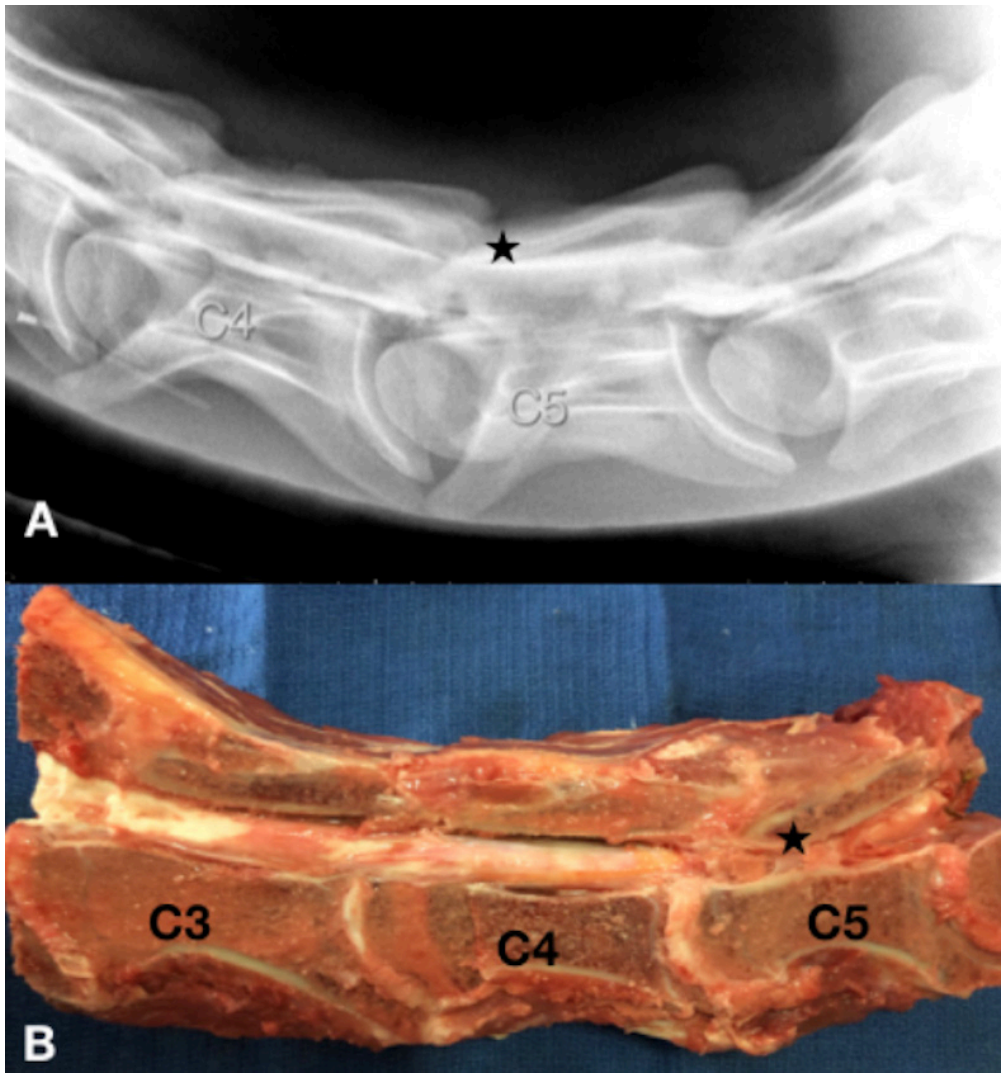

Figure 2. A) Contrasted cervical radiographs with neck in neutral position showing complete loss of the ventral dye column at C5. B) Anatomical dissection with longitudinal cross section of the C3-C5 cervical spine showing the site of spinal cord compression at C5 (black star).

this breed until today. The Caballo Raza Chilena horses are characterised by a lower growth rate and a muscled short neck conformation when compared to other breeds. It has been previously described that both rapid growth rates and length of the neck could be influencing the development of CVM (Rooney 1969, Levine *et al* 2008). Also, late taming and the start of training at approximately the age of 3 for this breed (Murúa 2006) results in a maintenance diet without overnutrition. All these factors could be involved in the low incidence of CVM in this breed.

In this case, the colt manifested classic signs of cervical spine impingement associated with ventral subluxation, also referred to as spondylolisthesis (Denoix *et al* 2005, Denoix 2007, Dyson *et al* 2020) between C4-C7. Also, a bony formation in the dorsal laminae of T1, probably as a result of retained cartilage matrix spicules (Janes *et al* 2015), along with a flare of the cranial vertebral epiphysis of T1 was noticed postmortem. Spondylolisthesis refers to a displacement of one vertebra on another (Denoix 2007) and it has been identified with transrectal ultrasonographic examination at the lumbosacral junction in adult horses (Denoix *et al* 2005), in the atlantoaxial joint in foals (Witte *et al* 2005) and recently in the cervical and cranial thoracic vertebrae associated with intervertebral disk disease in adult horses (Dyson *et al* 2020). Although the attenuation of dye at T1 during myelogram was attributed to the typical loss of dye column at that level, it is possible that these malformations and associated suspected impingement impeded the dye from progressing farther caudally beyond T1.

We were not able to delineate a specific cause for the cervical and thoracic malformations encountered in this horse. Abnormal *in-utero* development or position during pregnancy might have played a role in the development

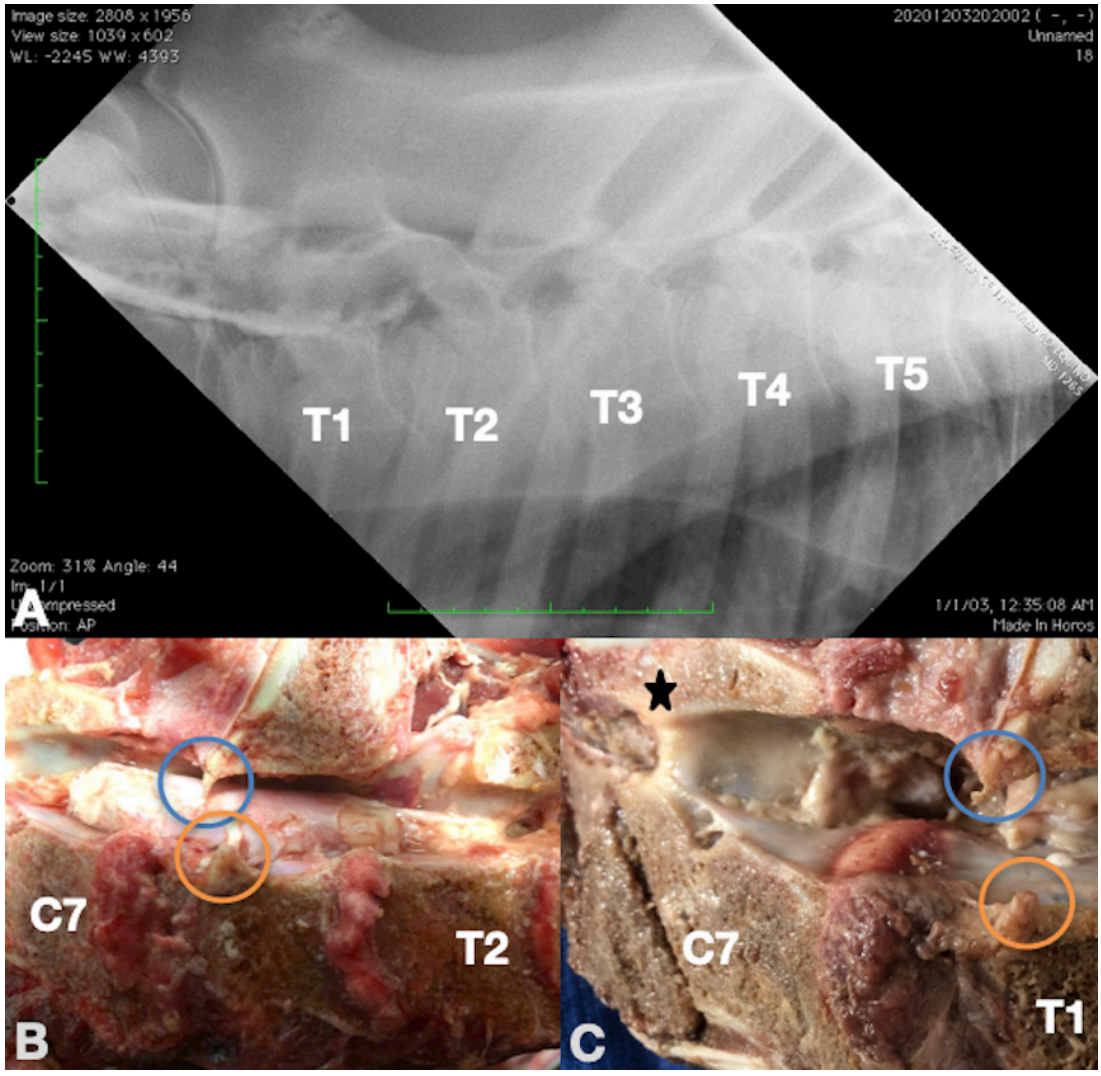

Figure 3. A) Radiograph taken during myelogram of proximal thoracic vertebrae (T1-T5). Contrast material is only observed until caudal T1. No thoracic abnormalities of bone were presumed from plain radiographs nor myelogram. B) Anatomical dissection with longitudinal cross section of the C7-T2 spine showing two large spicules, one in the cranial dorsal laminae of T1 (blue circle) and one in the cranial vertebral body of T1 (orange circle). C) Photograph showing spinal canal narrowing at C7 (black star) representing a second site of spinal cord compression.

of this condition, but neurologic deficits would have been expected to occur much earlier in the horse's life and no abnormalities were detected neither by the owner nor the field veterinarian before weaning. On the other hand, even though the diet was deemed adequate for this colt, it is not known to what extent trace nutrients, such as copper and zinc deficiencies, or calcium and phosphorus imbalance may have contributed to the presentation of the malformation observed in this horse. To our knowledge, there are no known calcium, phosphorus, copper, or zinc deficiencies in the area where the colt lived (Maldonado 2006). On the other hand, and even though blood and soil selenium concentrations were not measured, the south of Chile is characterised for being deficient in selenium (Crempien 1988, Tapia 2013) and as it is widely described that selenium is an essential microelement for animal development (Zarczyñska *et al* 2013, Hung Son and Duong Huyen 2019). However, similarly aged colts on the property, as well as direct relatives of the colt, were free from abnormalities. Despite the above, some degree of nutrient abnormality in the colt's diet cannot be ruled out nor can an underlying error of metabolism.

Radiographic indicators and measurements of the cervical canal height categorised by standard minimal sagittal diameter, and intravertebral and intervertebral ratios have been widely used despite the potential for false positive and false negative determinations of canal stenosis (Janes *et al* 2013). According to the above reference, spinal cord compression is possible if intra and intervertebral ratios are >50% for C4, C5 and C6 or >52% for C7 (Reed *et al* 2007), as shown in the colt presented in this case (table 1) in which the ratios were within normal parameters, but

spinal cord compression was only evident via myelography. For this reason, diagnosing sites of compression may be more accurate when both sagittal diameter ratios and myelographic measurements are used together (Hahn *et al* 2008). Additionally, postmortem magnetic resonance studies have shown that vertebral canal area and cord canal area ratios are better parameters to predict the location of cervical canal stenosis than only the sagittal plane of canal height (Janes *et al* 2013).

An important aspect that should be considered from this report is that radiographs and myelography can certainly underestimate the severity of spinal cord compression (Gough *et al* 2020). There are still some abnormalities that cannot be definitively diagnosed with conventional nor contrasted radiographs. Computed tomography (CT), or magnetic resonance are the imaging modalities that can appraise more accurately sites of spinal cord compression, vertebral structures, or bone changes and can help to confirm a specific diagnosis (Janes *et al* 2015, Gough *et al* 2020). Unfortunately, these advanced imaging tools are not available in many practices. Therefore, intra- and intervertebral sagittal ratios, dural diameter and contrast column reduction measurements often remain the only tools available to diagnose, prognose and propose treatment despite the risk of false positives or false negatives (Janes *et al* 2013).

Neurological clinical signs associated with cervical spinal cord compression were very characteristic in this case and considering the exacerbation of the signs with specific maneouvres, dynamic compression was presumed following clinical examination. However, the myelogram did not show significant differences in dye column reduction between changes in neck positioning (neutral, flexed or extended). Although no dynamic compression was evident during the myelogram, attenuation, and loss of contrast columns within vertebral bodies were observed in multiple sites, hence a static compression at C5, C6 and C7 was deemed more likely. Furthermore, even though the spinal cord compression was diagnosed after the myelogram, the severity of compression was only evidenced after necropsy (figures 2 and 3). Additionally, necropsy revealed a significant spicule in the cranial aspect of the dorsal laminae of T1 along with a flare of the cranial vertebral body of T1, neither of which were visible in the radiographs (figure 3). Most cases of thoracolumbar malformations described in horses have been manifested as gross deformity, usually without associated spinal cord compression and ataxia, and they include mainly transitional abnormalities and vertebral axis deviations (Lerner and Riley 1978, Denoix 2005). Thoracic vertebrae malformation causing neurological signs has been described only in a few horses before (Johnson *et al* 1997, Rush 2012). Even though thoracic spinal compression was not initially suspected after the myelogram, after correlating the clinical signs and necropsy findings, it is possible that the attenuation of dye at T1 represented compression at that level. Spinal cord histopathology would have further characterised the extent of compression but unfortunately, was not performed.

Although surgical treatments have been reported (Nixon 1991, Moore *et al* 1993, Kühnle *et al* 2018, Pezzanite *et al* 2021), further procedures were foregone in this case, and euthanasia was selected due to imaging findings, severity of clinical signs and associated poor prognosis.

Cervical malformation with spinal cord compression and ataxia is a common cause of neurological deficits in young horses of various breeds, while neurologic signs associated with thoracic vertebral malformation have been rarely reported. To our knowledge, this is the first reported case of multiple cervical spondylolisthesis and thoracic vertebral malformation with associated multi-level cord compression in the Caballo Raza Chilena horse. Vertebral malformation and malalignment should be considered as differentials for ataxia and neurologic deficits in young horses, including this Chilean breed, even if not evident radiographically.

ETHICAL STATEMENT

The owner agreed to the presentation and divulgation of this case report.

REFERENCES

Allison N, Moeller Jr. R. 2000. Spinal ataxia in a horse caused by an arachnoid diverticulum (cyst). *J Vet Diag Invest* 12, 279-281.

Bergmann W, Mik-van Mourik M, Veraa S, van de Broek J, Wijnberg I, Back W, Gröne A. 2020. Cervical articular process joint osteochondrosis in Warmblood foals. *Equine Vet J* 52, 664-669.

Cardona J, Betancur C, Alvarez J. 2013. Síndrome de wobblers en un caballo. *Rev MVZ Córdoba* 18, 3411-3415.

Cole R, Taintor J, Hanson R. 2017. Atlanto-axial malformation in an adult Quarter horse gelding. *Can Vet J* 58, 923-925.

Cunha dos Santos F, de Lourdes Adrien Delgado M, Fernandes C, Nogueira C. 2014. Cervical extradural haematoma in an ataxic horse. *Equine Vet Educ* 26, 306-309.

Crempien C. 1988. Deficiencia de selenio en ovinos. *Investigación y Progreso Agropecuario (IPA), INIA, La Platina* 47, 33-34

Denoix JM. 2005. Thoracolumbar malformations or injuries and neurological manifestations. *Equine Vet Educ* 17, 191-194.

Denoix JM. 2007. Discovertebral pathology in horses. *Equine Vet Educ* 19, 72-73.

Denoix JM, Audigié F, Coudry V. 2005. A review of diagnosis and treatment of lumbosacral pain in sport and racehorses. *AAEP Proceedings* 51, 366-373.

Derouen A, Spriet M, Aleman M. 2016. Prevalence of anatomical variation of the sixth cervical vertebra and association with vertebral canal stenosis and articular process osteoarthritis in the horse. *Vet Radiol Ultrasound* 57, 253-258.

Dörner C, Uzal F, Carvallo F, Palmero J. 2015. Compressive myelopathy caused by epidural haemotoma associated with fibrocartilaginous embolism in a horse. *Equine Vet Educ* 27, 405-409.

Dyson S, Bulsoni V, Salciccia A. 2020. Intervertebral disc disease the cervical and craneal thoracic vertebrae in equine: eight cases. *Equine Vet Educ* 32, 437-443.

Furr MO, Anver M, Wise M. 1991. Intervertebral disk prolapsed and diskospondylitis in a horse. *J Am Vet Med Assoc* 198, 2095-2096.

Gough S, Anderson J, Dixon J. 2020. Computed tomographic cervical myelography in horses: Technique and findings in 51 clinical cases. *J Vet Intern Med* 34, 2142-2151.

Grant B, Paterson J. 2006. How to perform a myelogram in less than 30 minutes. *AAEP Proceedings* 52, 74-77.

Hahn C, Handel I, Green S, Bronsvoort M, Mayhew I. 2008. Assessment of the utility of using intra- and intervertebral minimum sagittal diameter ratios on the diagnosis of cervical vertebral malformation in horses. *Vet Radio Ultra* 49, 1-6.

Henneke D, Potter G, Kreider J, Yeates B. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J* 15, 371-372.

Hirsch J, Grant B, Linovitz R, Peppers R, Rantanen T. 2009. Diagnosis and surgical treatment of epidural neoplasm in two ataxic horses. *Equine Vet Educ* 21, 564-568.

Hung Son N, Duong Huyen N. 2019. Selenium in aninal nutrition: metabolic pathways and animal response. *J Anim Sci Technol* 96, 2-13.

Janes J, Garrett K, Mcquerry K, Pease A, William N, Reed S, *et al*. Comparison of magnetic resonance imaging with standing cervical radiographs for evaluation of vertebral canal stenosis in equine cervical stenotic myelopathy. *Equine Vet J* 46, 681-686.

Janes J, Garrett K, Mcquerry K, Waddell S, Voor M, Reed S, *et al*. 2015. Cervical vertebral lesions in equine stenotic myelopathy. *Vet Pathol* 52, 919-927.

Johnson P, Johnson G, Pace L. 1997. Thoracic vertebral malformation in two horses. *Equine Vet J* 29, 493-496.

Kühnle C, Fürst A, Ranziger E, Sánchez-Andrade J, Kümmerle J. 2018. Outcome of ventral fusion of two or three cervical vertebrae with a locking compression plate for the treatment of cervical stenotic myelopathy in eight horses. *Vet Comp Orthop Traumatol* 31, 356-363.

Lerner D, Riley G. 1978. Congenital kyphoscoliosis in a foal. *J Am Vet Med Assoc* 172, 274-276.

Levine J, Ngheim P, Levine G, Cohen N. 2008. Associations of sex, breed, and age with cervical vertebral compressive myelopathy in horses: 811 cases (1974-2007). *J Am Vet Med Assoc* 233, 1453-1458.

Levine J, Scrivani P, Divers T, Furr M, Mayhew J, Reed S, *et al*. 2010. Multicenter case-control study of signalment, diagnostic features, and outcome associated with cervical vertebral malformation-malarticulation in horses. *J Am Vet Med Assoc* 237, 812-822.

Maldonado M. 2006. Contenido de nutrientes y metales pesados en suelos acondicionados con biosólidos, en experiencia de terreno y de laboratorio. *Memoria de título*. Universidad de Chile, Santiago, Chile.

MacMillan K, Bourque A, Perry L, Stoughton W. 2020. Acute non-ambulatory tetraparesis in a 6-month-old Standardbred weanling caused by a cervical vertebral epidural haematoma. *Equine Vet Edu* 33, 293-296.

Matthews H, Nout Y. 2004. Spinal cord, vertebral and intracranial trauma. In: Reed S, Bayly W, Sellon D (eds). *Equine Internal Medicine*. Philadelphia: D.C. Saunders, Pp 566-579.

Mayhew I, Watson A, Heisa J. 1978. Congenital occipitoatlantoaxial malformations in the horse. *Equine Vet J* 10, 103-113.

Moore B, Reed S, Robertson J. 1993. Surgical treatment of cervical stenotic myelopathy in horses: 73 cases (1983-1992). *J Am Vet Med Assoc* 203, 108-112.

Murúa JC. 2006. Evaluación del desempeño deportivo de caballos criollos chilenos en el rodeo chileno amansados por doma racional o doma tradicional. *Memoria de título*. Universidad Austral de Chile, Valdivia. Chile.

Nixon A, Stashak T, Ingram T, Norrdin R, Park R. 1984. Cervical intervertebral disk protrusion in a horse. *Vet Surg* 13, 154-158.

Nixon AJ. 1991. Surgical management of equine cervical vertebral malformation. *Prog Vet Neurol* 2,183-195.

Nout YS, Reed SM. 2003.Cervical vertebral stenotic myelopathy. *Equine Vet Edu* 15, 212-223.

Oswald J, Love S, Parkin T, Hughes K. 2010. Prevalence of cervical vertebral stenotic myelopathy in a population of thoroughbred horses. *Vet Rec* 166, 82-83.

Perris E, Reed S, Partington B, Getzy D. 1994. A block vertebra in an equine patient. *J Equine Vet Sci* 14, 664-667.

Pezzanite L, Easley J, Bayless R, Aldrich E, Nelson B, *et al*. 2021. Outcomes after cervical vertebral interbody fusion using an interbody fusion device and polyaxial pedicle screw and rod construct in 10 horses (2015-2019). *Equine Vet J* doi: 10.1111/evj.13449.

Piercy R. 2011. Cervical stenotic myelopathy. In: Ross M, Dyson S (eds). *Diagnosis and Management of Lameness in Horse*. W.B. Saunders, St. Louis, USA, Pp 649-654.

Reed S, Grant B, Nout Y. 2007. Cervical vertebral stenotic myelopathy. In: Furr M, Reed S (eds). *Equine Neurology*. Blackwell Publishing, Iowa, USA, Pp 283-298.

Rendle D, Durham A, Bestbier M, Smith K, Boswell J. 2008. Neurenteric cyst with associated butterfly vertebrae in a seven-month-old colt. *Vet Rec* 162, 558-562.

Rooney JR. 1969. Disorders of the nervous system In: Rooney JR (ed). *Biomechanics in lameness*. Williams and Wilkins, Baltimore, USA, Pp 219-233.

Rush B. 2012. Developmental vertebral anomalies In: Auer J, Stick J. editors. *Equine Surgery*, Philadephia: W.B. Saunders, p 693-699.

Sebastian M, Giles R. 2004. Fibrocartilaginous embolic myelopathy in a horse. *J Vet Med A* 51, 341-343.

Stewart R, Reed S, Weisbrode S. 1991. Frequency and severity of osteochondrosis in horses with cervical stenotic myelopathy. *Am J Vet Res* 52, 873-879.

Szklarz M, Lipinska A, Slowikowsa M, Niedzwied A, Marycz K, *et al*. 2019. Comparison of the clinical and radiographic appearance of the cervical vertebrae with histological and anatomical findings in an eight-month old warmblood stallion suffering from cervical vertebral stenotic myelopathy (CVSM). *BMC Vet Res* 15, 296.

Tapia J. 2013. Balance metabólico nutricional de selenio y variaciones estacionales de glutatión peroxidasa en equinos de la zona sur de Chile entre los años 2004 a 2011. *Memoria de Titulo,* Universidad Austral de Chile, Valdivia, Chile.

van Biervliet J, Mayhew J, de Lahunta A. 2006. Cervical vertebral compressive myelopathy: diagnosis. *Clin Tech Equine Pract* 5, 54-59.

Watson A, Mayhew I. 1986. Familial congenital occipitoatlantoaxial malformation (OAAM) in the Arabian horse. *Spine* 11, 334-339.

Witte S, Alexander K, Bucellato M, Sofaly C, Fife W, *et al*. 2005. Congenital atlantoaxial luxation associated with malformation of the dens axis in a Quarter Horse foal. *Equine Vet Educ* 17, 175-178.

Wong D, Scarratt W, Rohleder J. 2005. Hindlimb paresis associated with kyphosis, hemivertebra and multiple thoracic vertebral malformations in a Quarter Horse gelding. *Equine Vet Educ* 17, 187-191.

Zarczyñska K, Sobiech P, Radwiñska J, Rêkawek W. 2013. Effects of selenium on animal health. *J Elem* 2, 329-340.

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