Universidad Austral de Chile
Facultad de Ciencias Veterinarias

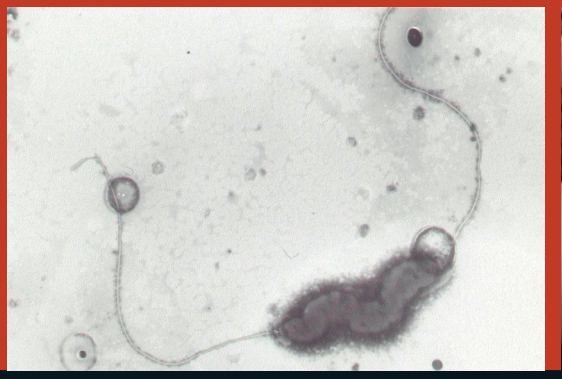
ORIGINAL ARTICLES

SHORT COMMUNICATION

CASE REPORT

Austral Journal of Veterinary Sciences

ISSN 0719-8000 / ISSN 0719-8132



VOLUME 52 / VALDIVIA - CHILE / 2020 / Nº 3

This journal is subsidised by
Dirección de Investigación y Desarrollo de la Universidad Austral de Chile (DID-UACh)

Periodicity: Triannual (January-May-September). Funded in 1969.
Indexed in:

– Current Contents Agriculture, Biology and Environmental Sciences (CC/AB and ES).
– Commonwealth Agricultural Bureau, International (C.A.B.I.).
– Dairy Science Abstracts.
– Veterinary Bulletin.
– Animal Breeding Abstracts.
– Helminthological Abstracts S.A.
– Agrindex.
– Biological Abstracts.
– Periódica.
– Focus on: Veterinary Sciences and Medicine.
– Science Citation Index Expanded.
– Scielo - Chile.
– Google Scholar.
– Ebsco.

ISSN 0719-8000
ISSN 0719-8132

Austral Journal of Veterinary Sciences

VOL. 52, Nº 3, 2020

Universidad Austral de Chile
Facultad de Ciencias Veterinarias
Casilla 567 - Valdivia - Chile

VOLUME 52, Nº 3, 2020

Austral J Vet Sci **52, 71-77 (2020)**

ORIGINAL ARTICLE

Are we at a turning point in journal assessment? An introduction to altmetrics

Erwin Krauskopf*

ABSTRACT. The status of any journal in which research is published is an important issue for academics. For many years the impact factor has been the criteria of choice to infer the quality of the research being published by each journal. However, with the massification of the internet, research currently impacts well beyond the academic community. This study aims to introduce readers into other bibliometric and non-bibliometric (altmetric) indicators that provide a wider perspective about the impact any particular research outcome may have besides citations. From a geographic viewpoint, the documents published by AJVS between 2010-2019 were written by authors affiliated to institutions from 33 different countries, mostly from Chile (37%) and Mexico (24%). These two countries served as collaboration nodes for countries from America, Asia, Europe and Oceania. From an altmetric perspective, 59 documents published between 2010-2016 were mentioned at least once in one of the sources tracked, being the majority of them through social media. Of particular interest is one document that was used as a reference for a patent issued in 2017 by researchers that were not related to the document published in the journal. Unfortunately, data for the documents published between 2017-2019 were unavailable, probably due to issues with the journal title change. Nevertheless, it is fair to conclude that since research outputs have shown to have an impact well beyond academia, it may be time to reconsider how journals should be assessed in the near future.

Key words: altmetrics, bibliometric, assessment.

INTRODUCTION

A past editorial of the Austral Journal of Veterinary Sciences (Anonymous 2019) introduced readers into the world of journal metrics based on the use of the journal impact factor to assess the quality of any given journal. Although many studies have expressed concerns with the misuse of the journal impact factor (JIF) (Colquhoun 2003, McKiernan *et al* 2019, Pang 2019), it is still used regularly to evaluate individual researchers, departments and institutions (Pan and Fortunato 2014). In Chile, the use of this indicator has even extended to the assessment of research proposals granted by the National Fund for Scientific and Technological Development (known as Fondecyt) in study areas such as "animal health and production"[1].

Opportunely, many members of the research community have considered alternative metrics to evaluate journals where to submit their manuscripts, following the guidelines set six years ago at the San Francisco Declaration on Research Assessment, also known as DORA (Pulverer 2013). This manifesto states that while the use of the impact factor as a promotional tool should be reduced, other journal metrics should be highlighted (such as editorial and publication times and h-index). One of the main issues about the impact factor is the fact that it can be influenced and biased. To estimate the impact factor for any given journal, the formula considers total citations received by all document types published during the two previous years, divided by the total number of articles and reviews published over the same two years. However, since citations are counted for document types not considered in the denominator (known as the numerator/denominator asymmetry), the impact factor is artificially inflated[2]. Furthermore, publishing a larger amount of reviews instead of other types of documents usually provides more citations to the journal. Other known strategies used to increase the citation rate of a journal consist of providing early access online to accepted manuscripts or by increasing its publication frequency. But in the end, the purpose of all high-quality journals should be the dissemination of new research findings to the precise audience, which is not only circumscribed to the academic world. In fact, the outcome of many published studies has benefited society or the environment as supporting evidence for clinical practice guidelines, systematic review and meta-analysis (including network meta-analysis) and also through the generation of public policies, patents, etc. For instance, the policy document "Salmonella in livestock production in Great Britain", issued by the UK government in 2016, was based on 26 documents that were published by different journals among which were Avian Pathology, Veterinarian Microbiology and Preventive Veterinary Medicine. Likewise, many documents published on scholarly veterinary journals have been used as reference

Received: 26.03.2020.

Accepted: 24.07.2020.

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[1] CONICYT. 2019. Bases Concurso Nacional de Proyectos Fondecyt Regular 2020. http://www.conicyt.cl/fondecyt/files/2019/05/Bases-Concurso-FONDECYT-Regular-2020.pdf; accessed January 2020.

[2] Lariviere V, Sugimoto CR. 2018. The journal impact factor: A brief history, critique and discussion of adverse effects. In: *Springer handbook of science and technology indicators*, Pp 1-33. https://arxiv.org/1801.08992.pdf; accessed January 2020.

for patents. As an example, an article that characterised proteases involved in egg hatching of the sheep blowfly (Young *et al* 2000) was used to generate a patent entitled "Methods and compositions for controlling ectoparasites" in 2012 (Application EP-2457582-A1).

Indeed, the massification of the Internet in the 1990s generated the possibility of evaluating the visibility of academic publications through new indicators, known as alternative bibliometric indicators (or altmetrics) (Priem *et al* 2012). These indicators certainly complement the information provided by traditional bibliometric indicators, allowing a more global assessment of the impact of scientific publications generated as a product of research projects. The main advantage of alternative bibliometric indicators is that these provide information at the article level, allowing their assessment well beyond academia, considering social, cultural, environmental, and economic returns of the research output (Anonymous 2018). Likewise, these indicators reflect in real-time if the article is discussed in social networks or other platforms (Zahedi *et al* 2014). One of the tools used to study these indicators is Altmetric explorer, which provides an Altmetric Attention Score (AAS) for all documents that have been mentioned at least once on the data sources tracked (table 1). The final score is derived from an algorithm that represents a weighted count of the amount of attention received by a specific research output[3].

Concomitantly, the purpose of this study was to assess the performance of the Austral Journal of Veterinary Sciences between 2010 and 2019, through the use of different bibliometric strategies, providing a journal assessment that moves away from the traditional impact factor.

MATERIAL AND METHODS

Bibliometric information was retrieved from Web of Science on the week of January 21, 2020 for the journal Austral Journal of Veterinary Sciences (and its previous title *Archivos de Medicina Veterinaria*) for the period 2010-2019. The downloaded data was sorted and processed using Microsoft Excel and SequelPro. The bibliometric map was built using the VOSviewer software (van Eck and Waltman 2010) based on the Web of Science downloaded data. To build the collaboration network, the information provided in affiliation records of each publication was utilised to extract the countries of co-authors.

Non-bibliometric information was retrieved using Altmetric Explorer[4] as the search engine because it captures real-time mentions in public policy documents, mainstream media, online reference managers, peer-review platforms and blogs (Altmetrics 2019, Hassona *et al* 2019). The query was made on January 22, 2020 for documents that were published by the journal titles

Table 1. Sources currently tracked by Altmetrics for mentions of research outputs* (Altmetrics 2020).

Source name	Weight	Notes
News	8	Manually curated news sources, with data provided via a third-party provider and RSS feeds direct.
Blogs	5	Manually curated list, harvesting links to scholarly content.
Policy documents	3	Scanning and text-mining policy document PDFs for references, which are looked up in CrossRef/PubMed and resolved to DOIs.
Wikipedia	3	Mentions of scholarly outputs collected from References section. English Wikipedia only.
Patent Citations	3	Scanning JSON patent records for links to publications and DOIs.
Twitter	1	Demographics, support for retweets, with monitoring of suspicious activity.
Post-publication peer reviews	1	Peer review comments collected from item records and associated by unique identifier.
F1000Prime recommendations	1	Scan for links to scholarly outputs.
Open Syllabus	1	Link syllabi's contents to HLOM IDs.
Facebook	0.25	Posts on public Facebook Pages only, with prioritised popular Pages.
Reddit	0.25	Includes all sub-reddits. Original posts only, no comments.
Q&A (Stack Overflow)	0.25	Scan for links to scholarly outputs.
YouTube	0.25	Scan for links to scholarly outputs in video descriptions.
Mendeley	0	Reader counts is number of readers with the output in their Library.
Web of Science Citations	0	Citation counts from peer-reviewed literature.
Dimensions Citations	0	Match outputs based on scholarly IDs.

* Altmetrics. 2020. What outputs and sources does Altmetric track? https://help.altmetric.com/support/solutions/articles/6000060968-what-outputs-and-sources-does-altmetric-track-; accessed June 22 2020.

[3] Altmetrics. 2019. Standards in Altmetrics. https://www.altmetric.com/about-altmetrics/standards-in-altmetrics/; accessed January 28, 2020.

[4] http://www.altmetric.com

"*Archivos de Medicina Veterinaria*" or "Austral Journal of Veterinary Sciences". It should be noted that Altmetric began collecting data in 2011.

RESULTS

A total of 440 documents were published by AJVS during the 10-years period, receiving 981 citations at the time the data was collected. While the majority of these documents were "article"-type documents (387 in total), the mean number of citations per review (26 in total) exceeded that of articles (5.6 *vs* 2.1 citations per document, respectively). Since the formulas to calculate the JIF and the Scimago Journal Rank (SJR) consider the total amount of citations as well as document types, this information is relevant for the editorial team while assessing journal performance. However, citations statistics only provide a rough measure of research impact.

An analysis of the geographic representation of the documents published by AJVS revealed that the authors were affiliated to institutions from 33 different countries. While Chilean authors have led in terms of contributions (37.0%) to the journal, the number of documents published annually has decreased over the years (figure 1). Interestingly, AJVS has been continuously used by Mexican researchers to publish their studies. In fact, only two out of the 106 documents published by Mexican researchers were in collaboration with Chilean institutions.

But how do researchers from these countries interact? As figure 2 depicts, Mexico has served as a node for studies in collaboration with countries such as the United States, England, Canada, Nigeria and Iran. Likewise, Chile is a junction in terms of collaboration with researchers from Europe, Oceania and America. A closer look at the affiliations registered by the authors of these documents revealed that the top five institutional contributors were Universidad Austral de Chile (Chile), followed by Universidad de Chile (Chile), Universidad de Caldas (Colombia), Universidad Nacional Autónoma de México (Mexico) and Universidad Autónoma de Baja California (Mexico). This information is relevant to readers as it confirms the international visibility achieved by the journal in the last 10 years.

A systematic search conducted on Altmetric Explorer for documents published by AJVS during the 10-years period revealed a total of 59 documents. The best scoring article (table 2) discussed the methods by which swine are slaughtered commercially from an animal welfare perspective. The breakdown of the AAS showed that 34% of the people that mentioned this article on Twitter were from Spain, followed by members of the public from Mexico (4%) and Argentina (3%). Furthermore, this article was bookmarked by 7 Mendeley readers and cited once by an article published in 2019 by the journal Meat Science. It has been suggested that Mendeley reader counts could be used to assess the future impact of a specific article since these accumulate earlier than citations (Maflahi and Thelwall, 2018). As shown in table 2, other documents published by AJVS additionally received Facebook mentions, which consist of Facebook shares, likes and comments on public Facebook pages that reflect the interactions users may have with a particular research.

In this context, the document entitled "*Conductas no deseadas en equinos*" published in 2010 is a good example of the impact a research output may have outside the academic community. Five years after the document was published by AJVS, the Venezuelan Veterinary Services of Integral Livestock posted on Facebook a link to the published document. One year later, a private

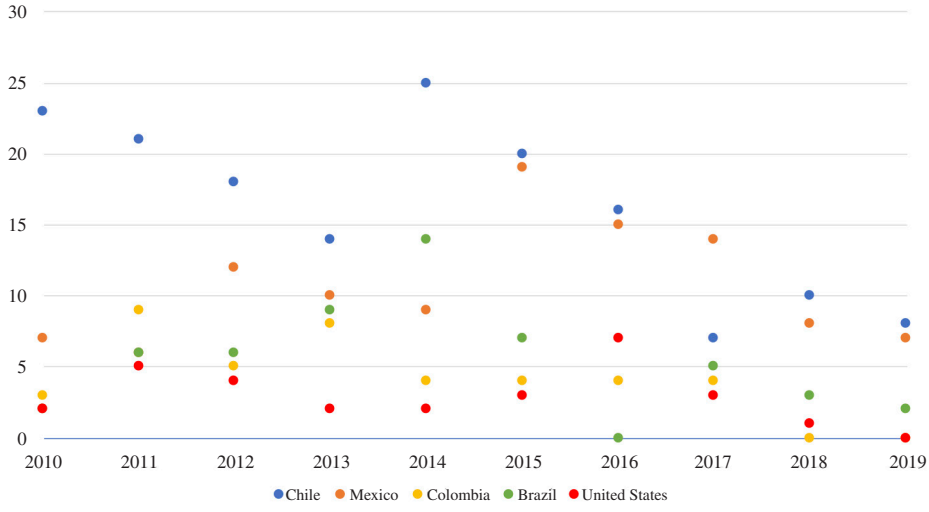

Figure 1. Total amount of documents published between 2010-2019 by researchers from the top-five most prolific countries.

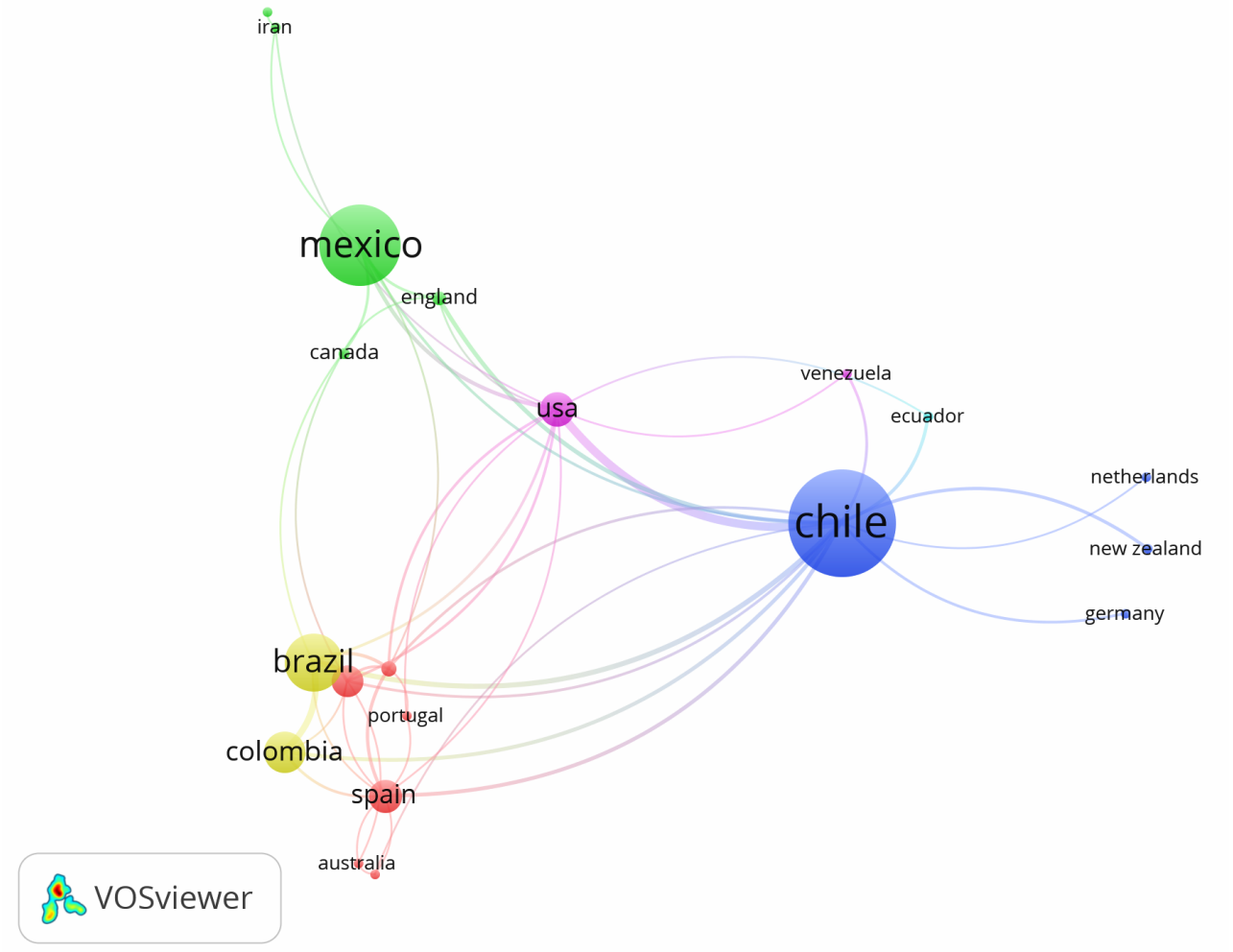

Figure 2. Collaboration network map. The size of the node (circle) represents the number of documents published by authors from that country. A line between two country nodes indicates that researchers from these countries published a document together. The thickness of the line represents the number of collaborations between two nodes.

pay-per-view TV channel from Colombia (TVAgro) posted on its YouTube channel[5] a video about the inappropriate behaviour observed during the treatment of horses which mentioned the AJVS study.

Another important aspect about altmetrics is that it tracks patent citations from the following jurisdictions: World Intellectual Property Organization, IP Australia, German Patent and Trademark Office, Swiss Federal Institute of Intellectual Property, European Patent Office, United States Patent and Trademark Office, French National Industrial Property Institute, Intellectual Property Office of the United Kingdom and the Netherlands Patent Office. From this perspective, one document published by AJVS was used as a reference for a patent even though it has only been cited three times since its publication in 2015. The document entitled "Distribution, epidemiological characteristics and control methods of the pathogen *Nosema ceranae* Fries in honey bees *Apis mellifera* L. (Hymenoptera, Apidae)" served as a reference for a patent granted in Chile entitled "Composition, method and use for controlling fungal infection caused by *Nosema ceranae* fungus in *Apis mellifera* bee colonies, comprising application, as a syrup (pj-cd14) and aerosol (pa-cd14), of an effective quantity of essential oil (cd14) obtained from *Cryptocaria alba* (peumo) leaves" (application WO-2017091915-A1). It is interesting to note that while this patent was filed by researchers from Universidad de Chile, the document published by AJVS was written by researchers from Universidad Católica de Temuco and the Agriculture and Livestock Service (SAG) from Chile. It seems that Chilean researchers are not imbued with the idea of needing to protect their discoveries even though a significant proportion of the new knowledge produced has commercial value (Krauskopf *et al* 2007, Sargent and Matthews 2014).

[5] https://www.youtube.com/watch?v=grXN0TJQmz4

Table 2. Top-10 research output ordered according to their Altmetric Attention Score (AAS).

Rank	Title	Year	AAS	# Tweeter mentions	# Mendeley readers	# Facebook mentions	WoS citations
1	*Evaluación de la eficacia del método de insensibilización por electronarcosis en porcinos*	2014	78	92	7	0	1
2	*Indicadores de bienestar animal para detectar problemas en el cajón de insensibilización de bovinos*	2012	27	32	25	1	12
3	*Evaluación de la oferta de pradera y tipo de concentrado sobre algunos parámetros ruminales en vacas lecheras en pastoreo otoñal*	2012	27	31	1	1	6
4	*Use of chitosan and polypropylene for the surgical correction of penile deviation in bulls: clinical and histological aspects*	2012	21	24	1	4	0
5	*Presencia del síndrome de úlcera gástrica en equinos de la policía militar*	2012	16	18	17	1	4
6	*Factores genéticos que inciden en la resistencia a enfermedades infecciosas en salmónidos y su aplicación en programas de mejoramiento*	2010	7	0	25	0	24
7	*Conductas no deseadas en equinos*	2010	5	5	67	1	6
8	*Distribution, epidemiological characteristics and control methods of the pathogen Nosema ceranae Fries in honey bees Apis mellifera L. (Hymenoptera, Apidae)*	2015	3	0	23	0	3
9	*Effect of the application of stem cells for tendon injuries in sporting horses*	2012	3	0	12	0	1
10	*Primer reporte en Chile de Chrysomya albiceps (Diptera: Calliphoridae) en evidencia entomológica forense*	2013	2	1	33	3	3

WoS: Web of Science.

DISCUSSION

Since 1969, AJVS (*formerly known as Archivos de Medicina Veterinaria*) has been published uninterruptedly. This timeliness, as well as its editorial content and the application of a peer-review process to select manuscripts, made the journal eligible to be indexed by databases such as Scopus (owned by Elsevier) and Web of Science (owned by Clarivate Analytics). Undoubtedly, the inclusion of any journal into these databases increases exponentially the citation rate of the authors (Krauskopf 2018). Furthermore, it fosters collaboration by displaying the expertise of researchers and the quality of their studies.

For journals that are editorially managed by universities, it is important to avoid publishing more than 20% of manuscripts authored by researchers from the same home institution (Fuentes *et al* 2013). Since most local and global rankings consider institutional research output, it seems logical that universities would benefit from publishing documents authored by their own researchers. A recent study determined that out of 22 Chilean journals managed by universities, 11 surpassed the 20% threshold, among them AJVS with 22.3% (Krauskopf 2020). Going into more detail, from the 85 documents that included at least one author from Universidad Austral de Chile, 42 were authored exclusively by researchers from Universidad Austral de Chile, 20 were the product of a collaboration between researchers from Universidad Austral de Chile and one or more Chilean institutions, and 23 included researchers from international institutions in collaboration with researchers from Universidad Austral de Chile and other national institutions. It is important to consider that publishing over the 20% threshold could limit the scope of intellectual coverage, reducing the geographical reach of the journal (Krauskopf 2020).

One of the unexpected results of this study was that contributions from Anglophone countries have not increased even though AJVS modified the language of publication and its name at the end of 2016, turning it into a completely English-language journal. Moreover,

the proportion of manuscripts authored by researchers from anglophone countries was 8.1% between 1994-2013, a time at which the proportion of English documents published by AJVS was 14.0% (Krauskopf *et al* 2017). It is important to bear in mind that AJVS is currently competing with 141 veterinary journals, according to the Journal Citations Report 2018, most of which publish in English. Thus, the problem might be that researchers from anglophone countries may not be aware of AJVS. Since we are in the era of digital sources, many researchers rely on online keyword searchers such as Google Scholar or by performing queries in social networks such as twitter. A study from 2016 that analysed twitter activities from undergraduate students and academics revealed very interesting results, such as that 73.6% of academics used twitter to search for information, 88.5% to share information, and 65.4% for academic reasons (Knight and Kaye 2016). In the case of undergraduate students, 65.7% used it to seek information and 27.7% for academic reasons. Although this study was limited to one institution from the United Kingdom, it shows how digital sources are playing a fundamental role in academic activities. Perhaps the time has come for AJVS to incorporate social networks as part of a digital strategy to create awareness about its contents to the world. Indeed, such an action would bring an increase in AAS.

With regard to alternative indicators, this study established that only 13.4% of the documents published by AJVS were picked up by Altmetrics. It is important to note that Altmetrics tracks mention of research outputs through the Digital Object Identifier (DOI) associated to each document. Regrettably, according to data extracted from the Web of Science, a total of 120 documents published by AJVS between 2010-2019 lack a DOI (figure 3). Other reasons why Altmetrics may have failed tracking more documents from AJVS might be more of a technical nature. For instance, since the Altmetrics text mining system requires access to data, the blogs need to include a research output identifier to detect a document. In the case of social media, an important issue is that Altmetrics only tracks public Facebook and Twitter accounts, simply due to access restrictions.

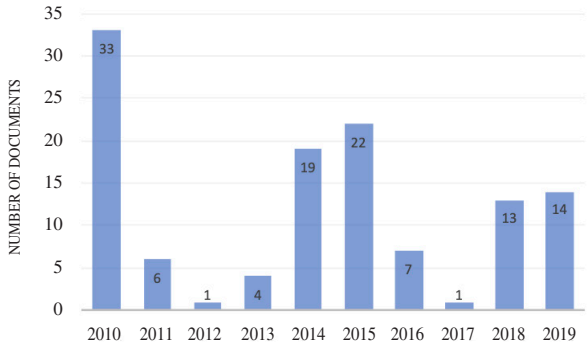

Figure 3. Number of documents published lacking a DOI (Digital Object Identifier).

It is important to note that there are some limitations to this study. First, concerns have been raised about the manipulation of Altmetrics. While this issue is of the essence, it is important to emphasise that this weakness is also seen on other scholarly metrics that are commonly used to assess research impact (Bartneck and Kokkelmans 2010, Krauskopf 2013, Delgado Lopez-Cozar *et al* 2014). Second, Altmetrics may be misinterpreted as no qualitative assessment of the Altmetric score is made. Mentions of a specific research on Twitter and Facebook may be positive, neutral or negative, just as it occurs with positive and negative citations. (Catalini *et al* 2015, Bai *et al* 2017). For instance, an article exhibiting a high AAS might be receiving a lot of online attention because the research presented is questionable. Lastly, as Altmetrics still remains at an ongoing state of development, the lack of benchmarks hinders AAS interpretation. In this context, Thelwall (2017) has proposed a strategy to estimate a set of field normalised alternative indicators. Currently, some research groups are developing frameworks to attend these issues (Fang *et al* 2020, Kassab *et al* 2020).

In conclusion, global access to the world-wide-web has changed scholarly communication forever. The discussion of research papers that was once restricted to the academic environment has moved onto scholarly social networking sites, making the information available for anyone interested to share and use. However, Altmetrics ought to be used as a complement of traditional metrics such as citation counts to assess research impact within the scholarly community and beyond. According to the Australian Research Council[6], research impact is defined as "the demonstrable contribution that research makes to the economy, society, environment and culture beyond the contribution to academic research". Perhaps it is time that we begin assessing our journals based on other parameters besides the impact factor.

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Austral J Vet Sci **52, 79-85 (2020)**

ORIGINAL ARTICLE

Knowledge, attitude, and practices of cattle farmers regarding zoonotic diseases in Erzurum, Turkey

Hayrunnisa Özlü[a], Mustafa Atasever[b], Meryem Aydemir Atasever[b]

ABSTRACT. This study aimed to determine the knowledge, attitude, and practices of cattle farmers regarding zoonotic diseases in Erzurum, Turkey, where cattle-raising is the most common occupation. A cross-sectional study was conducted on 1,045 cattle farmers in Erzurum. In terms of the diseases that can be transmitted from animal to human, 69.6% of the cattle farmers had information on anthrax, 62.8% on brucellosis, 18.4% on tuberculosis, 44.9% on rabies, 32.5% on Crimean-Congo hemorrhagic fever, 8.9% on hydatid cyst, 8.0% on toxoplasmosis and 7.9% on giardiasis. The knowledge level of cattle farmers who were university graduates was 94.8%. Cattle farmers having over 100 cattle had a knowledge level of 96.7% on zoonotic diseases and their positive attitudes and practices reached 95.1% and 91.8%, respectively. The results showed that the increase in education status, size of the enterprise, and monthly income of cattle farmers was related to an increase in knowledge, attitude, and practices regarding zoonotic diseases. However, it was found that the positive knowledge and attitudes of the cattle farmers could not be transformed into positive practices evenly.

Key words: zoonotic disease, cattle farmer, public health, knowledge level.

INTRODUCTION

Zoonotic diseases (also known as zoonoses) are caused by pathogens that spread between animals and people[1]. On a global scale, an estimated 60% of known infectious diseases and up to 75% of new or reemerging infectious diseases are zoonoses (Salyer *et al* 2017). Zoonotic diseases are known to be the cause of nearly 2.5 billion people getting sick per year and lead to 2.7 million deaths among sick ones annually (Magwedere *et al* 2012).

Domestic and wild animal population movements have a role in the occurrence and spread of zoonoses (Shanko *et al* 2015). On the other hand, the trend in increasing urban green spaces and spatial expansion of urbanised areas into agricultural and natural habitats also increases the dispersal and abundance of vectors into urban areas and their contact with humans (Tomassone *et al* 2018). Moreover, unofficially traded animals are a much greater risk factor for disease spread because they are not necessarily subject to veterinary controls (Fèvre *et al* 2016). The animals with sub-clinic infections disease cannot be distinguished from healthy animals and, therefore, diseases can be carried into different regions by animal movements (Hardstaff *et al* 2015). Some occupations require contact with animals and one of these is livestock farming. Livestock farmers are particularly exposed to zoonotic risks. Additionally, the lack of basic knowledge level, biosecurity precautions, and personal hygiene of livestock farmers can play a part in the infection and spread of zoonotic diseases (Weese *et al* 2002, Cediel *et al* 2012). Within the concept of activities occurring in the interface of human, animal, and environment, livestock farmers who have knowledge about zoonotic diseases can act more willingly in taking precautions and attending disease control programs.

Zoonotic diseases are important public health issues worldwide, including Turkey. Turkey, its geographical location and structure are exposed to many zoonotic diseases, including 107 different zoonotic infections such as Anthrax, brucellosis, rabies, Crimean-Congo hemorrhagic fever (CCHF) or tularemia (İnci *et al* 2018). Other zoonotic diseases investigated in this study, except for toxoplasmosis with a low incidence in cold areas, are seen much more frequently in intensive livestock farming regions in Turkey such as East Anatolia, Central Anatolia and Southeastern Anatolia (Ministry of Health 2011).

The East Anatolia Region, with 21% of the total area of Turkey, has crucial potential in terms of agriculture and livestock (Ateş and Terin 2008, TUIK 2018). Erzurum is the biggest province of the East Anatolia Region regarding surface area, and also has the highest amount of cattle. With 768,997 cattle, it is the second province of the country after Konya province in terms of the number of cattle (TUIK 2018). While some of the animals bred in the city are being used in animal production (Ünal 2011), about 20% are sold alive out of the city[2]. In this city where livestock farming is performed heavily, cattle farmers play an integral role in implementing zoonotic diseases prevention and transmission. Their knowledge level on zoonotic diseases,

Received: 01.04.2020.

Accepted: 14.08.2020.

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[1] CDC, Centers for Disease Control and Prevention. 2020. Zoonotic diseases. Available at: https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html; last accessed 15.06.2020.

[2] Anonymous. 2018. Republic of Turkey Ministry of Agriculture And Forestry Livestock Information System. Available at: https://hbs.tarbil.gov.tr

attitude towards risks associated with livestock production and hygiene practices may affect disease risks to humans and animals. In this sense, very few studies have been conducted in the region with a local base (Çakmur *et al* 2015, Kuşaslan Avcı *et al* 2017), and no studies had been carried out in Erzurum on this topic until now. This study aimed to determine the knowledge, attitude and practices of cattle farmers toward zoonotic diseases occurring in the city of Erzurum, Turkey, where animal breeding and livestock movement are performed intensely.

MATERIAL AND METHODS

This cross-sectional study was conducted between January 2016 and July 2017 on cattle enterprises located in 20 districts of Erzurum, Turkey. The province is the fourth largest city of Turkey and is located in the Eastern Anatolia region. Nearly 11% of Turkey's meadow and grasslands are in Erzurum.

The universe of the study consisted of 50,000 cattle enterprises located in 20 districts of the Erzurum province according to the 2016 Statistics of Animal Husbandry of Turkish Statistical Institute. Assuming that 50% of the farmers would have knowledge about zoonotic diseases, the sample size was calculated as 1,045 with confidence 95% and sampling error 0.03%. Questionnaires were handed out to enterprises selected using the random sample method and they were administered only to cattle farms, farmworkers and volunteers.

Data were collected using a questionnaire form that included 30 questions covering from demographic information to knowledge, attitudes and practices of cattle farmers regarding zoonotic diseases. The demographic information form included questions such as age, gender, number of children, educational status, monthly income and number of animals, and consisted of 10 questions. To evaluate the knowledge of the cattle farmers, certain questions were asked about important zoonotic diseases in Eastern Anatolia such as anthrax, brucellosis, tuberculosis, rabies, CCHF, toxoplasmosis, hydatid cyst, and giardiasis. Initially, the cattle farmers were asked if they had heard about the diseases transmitted from animals to humans and then they were told to identify which zoonotic diseases were transmitted to humans. The participants were informed of both the scientific and the local name of the diseases when doing the questionnaires. The questionnaire form containing the attitudes and practices related to zoonotic diseases included specific statements such as how do they dispose of animal waste, what protection equipment is being used when contacting animals, and what protective measures are being applied before consuming the obtained animal products.

Data were analysed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Percentage and frequency were examined as descriptive statistics. Pearson's chi-square was used to compare variables.

RESULTS

The study was conducted on 1,045 people aged between 18-79 years of which 1,015 (97.1%) were male and 30 (2.9%) were female. Out of the 1,045 cattle farmers who were the focus of the study, 134 (12.8%) of them were university graduates while 14 (1.34%) were illiterate. The number of married participants was 921 (88.1%), amongst them, 520 (56.5%) had less than five children and 13 (1.4%) had more than 10. The monthly income of the cattle farmers included in the questionnaire ranged between US$ 350 and US$ 8,500 (table 1).

It was established that 46.9% of cattle enterprises were dairy, 18.4% were fattening and 34.7% were mixed (dairy and fattening). The average cattle number was 39 and cattle farmers managed between three and 600 cattle. The number of enterprises with less than 10 cattle was 289, 329 enterprises had 11-30 cattle, 200 enterprises had 31-50 cattle, 151 enterprises had 51-100 cattle and 76 of them had over 100 (figure 1). One hundred and fifty of these enterprises were dealing with sheep and goat breeding at the same time.

To measure the knowledge level of the participants about zoonotic diseases they were asked the following question: "Do you have any knowledge about the diseases transmitted from animals to humans?", and 80.2% of the participants responded positively (figure 2). These participants were then asked: "Which of these infect humans: anthrax,

Table 1. Socio-demographic characteristics of the cattle farmers.

Characteristic	n	%
Sex		
Male	30	2.90
Female	1015	97.10
Age		
<25	47	4.50
25-40	276	26.41
41-65	691	66.12
65<	31	2.97
Education level		
Illiterate	14	1.34
Primary School	462	44.21
Secondary School	199	19.10
High School	236	22.60
University	134	12.82
Monthly income (US$)		
<850	532	50.90
850-1700	440	42.11
1700<	73	6.99

n: Number of cattle farmers.

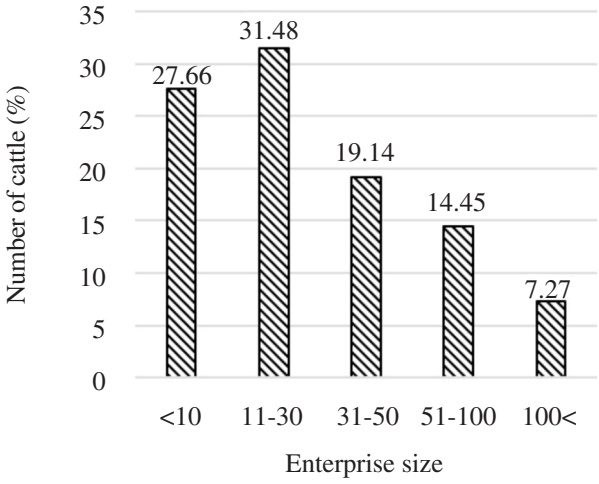

Figure 1. Enterprise size according to the number of cattle.

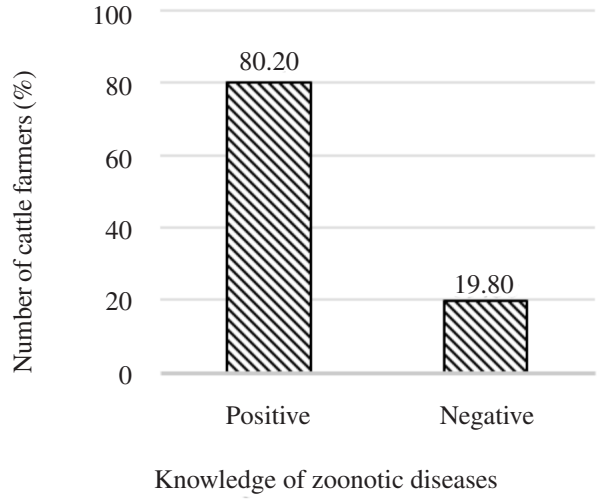

Figure 2. Knowledge level of cattle farmers related to zoonotic diseases.

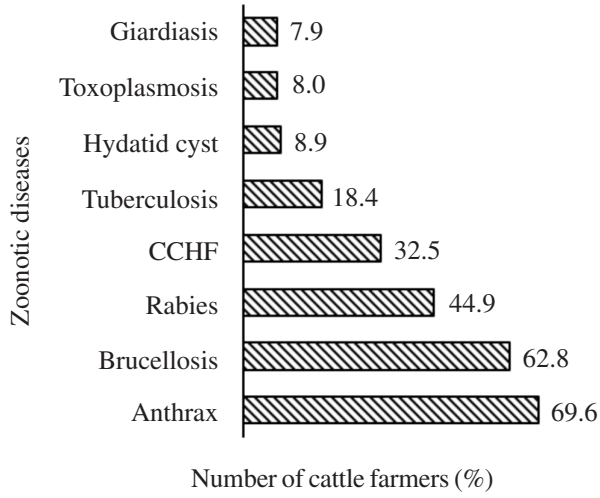

Figure 3. Knowledge level of cattle farmers related to the question "Which of these diseases infect humans?".

brucellosis, tuberculosis, rabies, CCHF, toxoplasmosis, hydatid cyst, or giardiasis?".

Regarding the infectious diseases transmitted from animals to humans, 69.6% (583) of the participants mentioned anthrax, 62.8% (526) brucellosis, 18.4% (154) tuberculosis, 44.9% (376) rabies, 32.5% (272) CCHF, 8.9% (75) hydatid cyst, 8.0% (67) toxoplasmosis and 7.9% (66) giardiasis (figure 3).

Table 1 shows the attitude and practices of cattle farmers toward zoonotic diseases. The question "Do animals need to be controlled regularly by a veterinarian?" was posed and 93.7% (979) of them responded positively. In the case of the question "Do you arrange regular veterinarian controls for your animals?" 90.3% (944) gave positive answers. However, when the frequency of this practice was asked, 33.8% (353) of them stated that they had their veterinarian control in two years period or more. Therefore, the answers of cattle farmers who scheduled their control one year or less were accepted as positive while the data was being evaluated. The farmers requesting regular veterinarian controls were 57.1% (597) (table 2).

Along with 73.2% (765) cattle farmers thinking that gloves should be used in animal contact, there were 56.1% (586) cattle farmers mentioning the necessity of using masks. Those who use gloves and masks while in contact with animals had a ratio of 65.8% (688) and 23.9% (250), respectively. Only 89.7% (937) of cattle farmers knew that they might be infected through a scar on the hand while contacting animals. It was detected that 19.8% (207) of cattle farmers continued to contact the animal with a scar on their hand. Regarding infected animals, 92.4% (966) of cattle farmers stated that the dead body of an infected animal should be buried under the soil deeply. The percentage of those who exterminate the dead body in this way was 90.7% (948). While 80.0% (836) of cattle farmers said that milk needed to be boiled at least 5 minutes, the ones who actually boiled the milk at least five minutes were 76.7% (802) (table 2).

There were differences between the positive knowledge level, attitude and practices of cattle farmers regarding zoonotic diseases and their educational level, enterprises size, and monthly income. The positive knowledge level of illiterate farmers about zoonotic diseases was determined as 65.7% whereas those who graduated from university had a positive knowledge level of 94.8%. On the other hand, despite the high knowledge level of university graduates, there was a decrease in the transformation of knowledge into positive practices as high as approximately 10%. Regardless of the size of enterprises, all cattle farmers had a high level of positive knowledge about zoonotic diseases. However, their positive attitude and practice ratios were

low. In enterprises having more than 100 cattle, 96.7% of cattle farmers had positive knowledge level, 95.1% had a positive attitude and 91.8% had positive practice. It was detected that there were differences in the positive knowledge, attitude, and practices of cattle farmers depending on their monthly income. The increase in monthly income was associated with an increase in the level of positive knowledge, attitude, and practices (table 3).

Table 2. The rates of positive attitude and practices of cattle farmers related to zoonotic diseases.

	Positive Attitude		Positive Practice	
	n	%	n	%
Getting veterinary support for treatment	982	94.0	947	90.6
Making regular veterinarian control	979	93.7	597	57.1
Washing hand	997	95.4	957	91.6
Using glove	765	73.2	688	65.8
Using mask	586	56.1	250	23.9
Wearing boot	903	86.4	903	78.3
Avoid contact with scary hands	937	89.7	838	80.2
Disposal of animal carcass	966	92.4	948	90.7
Boiling milk	836	80.0	802	76.7
Make cheese with boiled milk	908	86.9	813	77.8
Avoid raw meat eating	941	90.0	926	88.6

n: Number of cattle farmers.

Table 3. Distribution of factors affecting positive knowledge, attitude and practices of cattle farmers related to zoonotic diseases.

	Factors	Knowledge			Attitude			Practice		
		n (%)	χ^2	P	n (%)	χ^2	P	n (%)	χ^2	P
Education level	Illiterate	9 (64.3)	31.934	0.0001 ***	9 (64.3)	7.953	0.093	8 (61.1)	15.354	0.004 **
	Primary School	347 (75.1)			390 (84.4)			338 (73.2)		
	Secondary School	162 (81.4)			170 (85.2)			154 (77.4)		
	High School	203 (86.0)			206 (87.4)			195 (82.6)		
	University	127 (94.8)			120 (89.6)			113 (84.3)		
Size of enterprise (number of cattle)	<10	173 (77.6)	15.164	0.004 **	138 (69.5)	63.985	0.0001 ***	110 (61.9)	125.110	0.0001 ***
	10-30	318 (80.4)			352 (89.1)			322 (81.5)		
	31-50	168 (83.2)			182 (90.1)			172 (85.1)		
	51-100	142 (86.8)			150 (91.5)			143 (87.2)		
	100<	59 (96.7)			58 (95.1)			56 (91.8)		
Monthly income (US$)	<850	427 (80.3)	21.860	0.0001 ***	466 (87.6)	6.543	0.034 *	411 (77.3)	16.154	0.0001 ***
	850-1700	394 (89.5)			401 (91.1)			376 (85.4)		
	1700<	69 (94.5)			70 (95.9)			67 (91.8)		

*P<0.05, **P<0.01, ***P<0.0001.
n: Number of cattle farmers.

DISCUSSION

This study applied a broad concept in terms of the knowledge, attitude, and practices level of cattle farmers in Erzurum related to zoonotic diseases. Despite the knowledge level of cattle farmers about the diseases transmitted from animals to humans being high (80.2%), their knowledge level to identify which diseases are zoonoses was low. Three out of 8 zoonotic diseases included in the questionnaire were identified as zoonoses by the cattle farmers (anthrax, brucellosis, and rabies). As for hydatid cyst, toxoplasmosis, and giardiasis diseases, their knowledge level was low (figure 3). Cattle farmers had a high level of knowledge about some of the zoonotic diseases probably because of the impact of mandatory vaccination campaigns targeting those diseases, applied to all bovines by the authorities in Turkey.

In our study, 69.6% of the cattle farmers knew that anthrax was a zoonotic disease and 62.8% of them also knew brucellosis. However, these ratios were much higher in a study conducted in Kars (Çakmur *et al* 2015). These differences between cities can be explained by the prevalence rate. In Turkey, between 1995 and 2010, the prevalence rate of anthrax cases was much higher in Kars when compared to Erzurum (Ertek 2011).

Turkey is still an endemic region in terms of rabies, with approximately 250,000 rabies-risk contact reports being made annually, averaging one to two rabies cases per year (Anonymous 2019). In our study, 44.9% of the cattle farmers knew that rabies was a zoonotic disease. However, the level of knowledge was not as high as in those who knew that anthrax and brucellosis were zoonoses. In a study conducted in Erzurum, 73% of the cases in emergency services due to the risk of rabies contact came from urban areas (Can *et al* 2020).

It was reported that the first human case of CCHF in 2002 was in Central Anatolia, Turkey. A total of 11,040 cases were reported in Turkey between 2002 and 2018, and 528 of them died (Anonymous 2019). In our study, 32.5% of the cattle farmers knew that CCHF was a zoonotic disease. Studies conducted in different provinces of Turkey have reported that knowledge about CCHF was insufficient (Ozer *et al* 2008, Yılmaz *et al* 2009, Çilingiroğlu *et al* 2010).

Inadequate personal hygiene and farming sanitation during close contact with animals, infected animal slaughtering and skin striping, wrong extermination of sick animal waste, and infected material can cause zoonotic diseases (Tebug *et al* 2015, Rajkumar *et al* 2016). Therefore, the people involved in such activities with livestock are always under a high risk in terms of zoonotic diseases (Martin *et al* 2011, Musallam *et al* 2015). In the study, among the preventive methods from zoonotic diseases, over 90% of cattle farmers had both positive attitudes and positive practices regarding handwashing after the contact with an animal, the burial of dead animal bodies, and separation of sick animals from the herd (table 2).

Only for treatment of their animals, 90.6% of cattle farmers had veterinary services whereas 57.1% had regular veterinary controls for their cattle. In a study conducted in the Çayırlı district of Erzincan, east of Erzurum, it was reported that 73.3% of dairy farmers benefitted from veterinary services when their animals were sick, 6.6% of them had regularly veterinary controls (Özyürek *et al* 2014). In our research, the frequency of practices such as wearing boots inside the enterprise (78.3%) and using gloves while in contact with animals (65.8%) was higher than the practice of using a mask (23.9%) (table 2). Odo *et al* (2015) and Munisamy *et al* (2017) reported percentages related to the use of gloves (51% and 36.8% respectively) and masks (26.0% and 18.4% respectively) among livestock farmers that were lower than those in our research.

Production of animal products, contaminations during this production, wrong feeding habits and lack of knowledge can be effective in the transmission of zoonotic diseases (Tebug *et al* 2015, Rajkumar *et al* 2016). It must be highlighted that through the consumption of raw milk and milk products, several bacterial zoonotic diseases can infect people except for brucellosis and tuberculosis (Özlü and Atasever 2018). In this study, nearly ¾ of cattle farmers mentioned that they consumed milk after a minimum of five minutes of boiling and did not make cheese from raw milk. A research conducted on sheep and goat farmers in the Van province, located in Southeast Erzurum, and in the same geographical region (Eastern Anatolia Region), showed that most of the participants made cheese from raw milk and some consumed the cheese they made freshly. (Kuşaslan Avcı *et al* 2017). In a meta-analysis study done on the awareness level of brucellosis disease globally (21 studies), it was found that ratio of awareness was 44.5% that raw milk consumption for being a risk factor for brucellosis. (Zhang *et al* 2019). In our study, attitude and practice levels of cattle farmers regarding the topic of non-consumption of raw milk and cheese made from raw milk were high.

Although raw meat consumption has been applied for generations in many social groups located in Russia, Cuba and Africa (Abera *et al* 2016), there is no habit of raw meat consumption in our country. Raw meat consumption creates dangerous situations in terms of public health due to parasite diseases originated from food as well as bacterial diseases (Murrell 2013, Bintsis 2017). Although Erzurum is a city with high red meat consumption, 88% of cattle farmers included in the questionnaire answered that they did not consume raw meat. This situation showed similarity with the work of Çakmur *et al* (2015). Zoonotic diseases constitute 70% of community-acquired diseases.

In relation to the occurrence of these diseases, it is known that sociocultural habits and socioeconomic conditions have a strong influence (Dinçer *et al* 2003). If factors such as socioeconomic status of cattle farmers, their

education levels, and the enterprise sizes are considered, it is important to put forward the risks of enterprises related to zoonotic diseases, prevent these diseases and develop control strategies. In this research, no significant differences were found between "ages of the cattle farmers" and "their knowledge, attitude, and practices about zoonotic diseases" ($P>0.05$). However, there was a significant difference between "education levels, enterprise sizes, income levels" and "knowledge, attitude, and practices toward zoonotic diseases" ($P<0.05$).

When the knowledge and practice of cattle farmers related to zoonotic diseases were compared with their education status, there was a significant difference between groups ($P<0.01$), but if only their attitudes were compared, it was observed that there was no significant difference. It was confirmed that especially the ones who had high school and university education had a knowledge level distinctly higher and their attitude and practices, except for those who were not illiterate, were closely related to each other. In the same manner, studies conducted in Tajikistan, Senegal, Nepal, and India reported that livestock farmers with low education level had a low level of knowledge, attitude, and practices toward protection from zoonotic diseases (Lindahl *et al* 2015, Tebug *et al* 2015, Kelly *et al* 2018, Prasad *et al* 2019).

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Small scale livestock enterprises play a significant role as a source of income and feeding for low and middle-income countries (Herrero *et al* 2013). Small scale and poor livestock farmers are more often affected by animal diseases, which reduce their income and kill or weaken their animals, destroying much of their food resources and assets (Pradere 2014). In this study, as the number of animals increased in cattle enterprises, knowledge, attitude, and practice levels of cattle farmers also increased. It was also observed that there was a significant difference between groups ($P<0.01$). It was found that most of the cattle farmers included in the questionnaire had a high level of knowledge related to zoonotic diseases and also a high level of knowledge regarding the attitude causing the infection of these diseases but they did not transform that knowledge into practice evenly. Cattle farmers who had more than 100 cattle had knowledge, attitude, and practices toward zoonotic diseases at a level of over 90% and it was higher than other groups (table 3). At the same time, most of the enterprises with more than 100 cattle were having regular veterinary surgeon services. This situation decreases the cost of veterinarian service per unit as the number of animal increases. In any case, cost increase causes small and middle scale enterprises to benefit less from veterinarian service depending on the decrease in the animal count (Haan and Umali 1992).

Livestock farming composes at least 70% of poor people's mainstay who live in rural areas in the world (Schelling *et al* 2007). Animal production accounts for 64% of the agricultural economy of Erzurum and it is one of the primary sources of income for the community (Çoban *et al* 2013). In this study, when the monthly income of cattle farmers was compared with their knowledge, attitude, and practices toward zoonotic diseases, the difference was significant ($P<0.05$). It was observed that as the monthly income increased, cattle farmers' knowledge, attitude, and practice levels also increased. Nearly all of the enterprises having more than 100 cattle had a monthly income of over US$ 1,700 (table 3). In major scale enterprises, regardless of the count, one or more animals being sick can affect the health of all animals negatively. For this reason, cattle farmers are well aware of keeping a budget for taking veterinarian service in protection of animal health and treatment.

In Erzurum province, where cattle farming is performed intensely, the knowledge, attitude, and practice levels of cattle farmers increased depending on the increase in their education level, their enterprise size and their monthly income. However, regardless of education level, enterprise size and monthly income, it was determined that the positive application levels of all cattle farmers were always lower than the positive attitude levels. Further studies are needed to identify the reasons for the low level of positive practices among cattle farmers. Besides, to prevent and decrease the risks of zoonotic diseases throughout the city, cattle farmers should be given training on topics such as public health, animal health, food security, environment protection, personal cleaning and hygiene. By conducting such studies and providing training to livestock farmers, the opportunity to intervene in a number of zoonotic disease transmission cycles can be improved. It could also be applied to the One Health approach, i.e. public veterinary, environmental and human health function as part of an integrative system.

ACKNOWLEDGEMENTS

This work was supported by Atatürk University Scientific Research Project Unit (BAP ID:2765-PRJ2015/59).

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Austral J Vet Sci **52, 87-94 (2020)**

ORIGINAL ARTICLE

Genetic structure and population dynamics of autochthonous and modern porcine breeds. Analysis of the *IGF2* and *MC4R* genes that determine carcass characteristics

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ABSTRACT. To know the genetic situation of the Pampa Rocha, Celta, Bizaro Portuguese, Duroc, Iberian Extremeño and Iberian Andalusian porcine populations, their genetic structure and population dynamics were studied on the *IGF2* and *MC4R* genes, which determine meat characteristics and quality. The degree of genetic variability (He = 0.2511 in Pampa Rocha; 0.0278 in Celta; 0,1453 in Bizaro Portuguese; 0.3719 in Duroc; 0.0764 in Iberian Extremeño and 0.0384 in Iberian Andalusian), genetic distance, and the presence or absence of consanguinity were studied. The Fis values were positive for the Duroc population (0.00426) indicating a very low inbreeding, the rest of the populations did not present consanguinity. Significant deviations ($P \leq 0.05$) in the Hardy-Weinberg (HW) equilibrium were obtained for the *IGF2* locus in Celta, Iberian Extremeño, and Iberian Andalusian populations with the G allele fixed, while the Bizaro Portuguese, Pampa Rocha, and Duroc populations presented polymorphism, the G allelic frequency was much higher than A allele, except in the Duroc breed (0.15). These findings could help breeders to increase the presence of the A allele for the improvement of muscle mass and reduction in the back-fat thickness in this breed. All the studied populations presented polymorphism for the *MC4R* locus with different frequencies for each allele. Furthermore, these results could allow developing strategies against anthropogenic activities that hinder the conservation of the biodiversity of these porcine breeds.

Key words: pig populations, *IGF2, MC4R,* genes.

INTRODUCTION

The enormous scale at which porcine breeds are crossed to achieve greater production and better quality of meat has led to critical changes in the genetic structure of the various pig breeds farmed worldwide. Genetic variability has been drastically reduced and replaced by a genetic selection that seeks higher production. High rates of consanguinity, with the consequent loss of biodiversity, threaten these breeds. The knowledge of the genetics of swine breeds is important, especially for those genes that encode or regulate the traits desirable in the meat industry, as they are subject to high selection pressure (Andersson *et al* 1994, Azevedo *et al* 2015, De Campos *et al* 2015).

To identify the mechanisms that allow the maintenance of genetic variability, it is necessary to have suitable estimators of its magnitude, as well as to perform adequate characterisation of the patterns that are observed (Cockerham 1973). One method for the estimation of genetic variability is the use of DNA markers from each population (Avise 1994). These estimators of genetic populations allow us to determine whether genetic differences exist between two populations, identify if there is fragmentation in the population from a genetic perspective (population structure) and whether the identified variability can be explained by selection (Fisher 1930). However, in addition to selection, other effects can also affect the existing variability. For example, events that have occurred throughout history including drastic reductions in population size produced by a bottleneck such as an environmental disaster, the hunting of a species to the point of extinction, or habitat destruction that results in deaths (Fontdevila and Moya 1999). There is also the founder effect that occurs when a new population is established by a very small number of individuals from a larger population, the genetic drift that occurs when there is a change in the frequency of an existing gene variant (allele) in a population due to random sampling of organisms, and the mutations, among others. All of them can generate unexpected patterns in population parameters (Haldane 1932, Phillips *et al* 2019).

A population can quickly recover a high number of alleles if its structure is known and crosses are handled appropriately; however, it should be noted that if there are overlapping generations, as occurs in pig farms, the effect of the reduction of the population will be different. (Wellmann and Bennewitz 2019).

As indicated by González-Sarabia *et al* (2011), in some countries, such as Spain, Mexico, and Cuba, it has been observed that genetic variation in pig populations of autochthonous breeds is higher than that in commercial breeds. The autochthonous breeds are phylogenetically separated from the commercial breeds, which suggests that their genetic structures have been conserved owing to the

Received: 04.03.2020.

Accepted: 14.08.2020.

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lack of systematized breeding programs (Lemus-Flores *et al* 2001). Although no productive selection programs have been applied to these breeds, they have survived for more than 500 years and have a genetic reservoir to produce better-adapted national varieties (Lemus-Flores *et al* 2001).

Candidate genes associated with meat quality, such as the *IGF2, MC4R* genes (Fassa *et al* 2015), are excellent markers to identify the presence or absence of genetic variability in porcine populations.

Insulin-like factor 2 (*IGF2*) plays a role in muscle growth and proliferation and differentiation of myeloblasts. Van Laere *et al* (2003) discovered a polymorphism in the *IGF2* locus, which was localised in the regulatory region of the gene. As indicated by Fassa *et al* (2015), the 3072 G>A substitution in the third intron of this gene alters the binding site of a nuclear repressor by tripling the expression of mRNA in skeletal muscle during postnatal growth, when the allele A is inherited from the male parent (genomic seal). This event leads to an increase in muscle mass and reduction in the back-fat thickness (BF), but does not affect the quality of the meat (Carrodeguas *et al* 2005, Estellé *et al* 2005, Den Maagdenberg *et al* 2008, Oczkowicz *et al* 2012, Fontanesi *et al* 2010 and 2012, Burgos *et al* 2012). The authors also established the association of this polymorphism with other characteristics of economic importance, such as body weight, growth rate, and conversion efficiency. One of the main objectives of selection in pigs during the past decades has been to obtain fast-growing and lean animals. It is assumed that the rapid evolution in this direction has accompanied the selection of the beneficial allele A of the *IGF2* gene in the different porcine breeds. Oczkowicz *et al* (2012) studied the genotype and genotypic frequencies of different breeds in Poland, reporting 100% and 91% for the AA genotype in the Duroc Jersey and Pietrain breeds, respectively, and a frequency of 27% in the local Pulawska breed.

The melanocortin 4 receptor gene (*MC4R*) codes for a G protein transmembrane receptor with an important role in the control of energy homeostasis and is a component of the central melanocortin pathway. In mammals, this receptor is expressed mainly in the central nervous system in regions that control food consumption, body weight, and energy homeostasis (Krashes *et al* 2016, Shen *et al* 2017). In pigs, Kim *et al* (2000) found a missense mutation, 1426 A> G, in the sequence encoding the seventh transmembrane region of the melanocortin receptor that results in the replacement Asp298Asn. The G allele was found to be associated with a lower BF, slower growth rate, and lower consumption (Burgos *et al* 2006, Bruun *et al* 2006, Meidtner *et al* 2006, Van den Maagdenberg *et al* 2008, Piorkowska *et al* 2010, Davoli *et al* 2012, Fontanesi *et al* 2013, Samoré and Fontanesi 2016).

Therefore, knowledge of the genetic structure of these breeds and their dynamics is crucial to plan appropriate strategies for the protection of native breeds and the conservation of their genetic diversity in the future.

The objective of this work was to study population dynamics and genetic structure in the Pampa Rocha, Celta, Bizaro Portuguese, Duroc, Iberian Extremeño and Iberian Andalusian porcine breeds, based on the *IGF2* and *MC4R* genes, which determine meat characteristics and quality. These analyses allowed us to determine the degree of genetic variability, the genetic distance between the breeds, and presence or absence of consanguinity to help develop strategies against anthropogenic activities that hinder the conservation of the biodiversity of these breeds.

To protect native breeds, it is important to consider the specific characteristics of each one of them, along with the genetic divergence between them, and acknowledge the effect of exploitation exercised by man to achieve and maintain the necessary balance between the desired characteristics and their genetic specificity in a breed.

MATERIAL AND METHODS

ANIMALS

We studied 233 animals of six different breeds of pigs (58 Pampa Rocha, 35 Celta, 45 Bizaro Portuguese, 40 Duroc, 24 Iberian Extremeño, Spain, and 25 Iberian Andalusian, Spain). Six wild boars were used as control animals and several hunters supplied samples of frozen muscles from wild boars. Frozen muscle samples from Iberian pigs were obtained from COVAP (Pozoblanco, Spain) and CENSYRA (Badajoz, Spain). Dr. Carballo García (University of Vigo, Spain) provided frozen muscle samples from Celta pigs and hair samples from Bizaro Portuguese pigs. Hair samples from the Pampa Rocha pigs were obtained from animals belonging to the reproductive group of the Pig Production Unit of the South Regional Center of the Experimental Station of the Faculty of Agronomy (University of the Republic) located in Progreso, Canelones (Uruguay). Semen samples of the Duroc pigs were obtained from Agropecuaria OBANOS S.A. Marcilla (Spain).

The Bioethical Committee Certification has been obtained and all researchers who have collaborated in this work have the Technical Competence in Animal Health, Husbandry and Handling.

DNA EXTRACTION

DNA was extracted from muscle, semen, or hair follicles by using the Realpure Genomic DNA Extraction Kit[1] and the GenomicPrep™ DNA isolation kit[2] following the manufacturer's instructions. The concentration of DNA in the extract was determined by using a NanoDrop™

[1] Durviz (Valencia, Spain).

[2] Amersham Biosciences (Little Chalfont, United Kingdom).

1000 spectrophotometer[3]. DNA was extracted from all the samples reaching a DNA concentration of about 4 x 10^2 ng/µL and diluted to a working concentration of 1 ng/µL.

GENOTYPING

Genotyping for *IGF2* and *MC4R* was performed by real-time PCR as described by Carrodeguas *et al* (2005), Burgos *et al* (2006 and 2012), Galve *et al* (2013) and Latorre *et al* (2016). The PCR reactions were carried out using an ABI-PRISM 7000 apparatus[4].

For the *IGF2* gene amplification, each assay contained 12.5 µL of TaqMan Universal PCR Master Mix, 0.6 µL of 40x Assay Mix, 5 µL of genomic DNA (1 ng/µL) and double distilled H_2O to 25 µL. PCR was carried out using an initial cycle of 10 min at 95 °C followed by 45 cycles of 15 s at 92 °C and 1 min at 61 °C. The primers used were IGF2F (AGCCAGGGACGAGCCT) and IGF2R (GAGGCCCGCGGACTC). The probes were IGF2V1 (CTAGGCTCGCAGCGC, labelled with the dye VIC) and IGF2M1 (CTAGGCTCACAGCGC, labelled with the dye FAM).

For the *MC4R* gene, the PCR assay sets contained 12.5 µL of TaqMan Universal PCR Master Mix, 0.6 µL of 40x Assay Mix, 5 µL of genomic DNA (1 ng/µL) and double distilled H_2O to 25 µL. PCR reaction was carried out using an initial cycle of 10 min at 95 °C followed by 50 cycles of 15 s at 92 °C and 1 min at 60 °C. The primers used were PIGBRAIN-MC4RF (TGCTTCATGTCTCACTTTAATTTGTATCTCA) and PIGBRAIN-MC4RR (GGCTCCGGAGTGCATAAATCA). The probes were PIGBRAIN-MC4RV1, attached to VIC fluorescent label, (CATCATCGATCCCC) and PIGBRAIN-MC4RM1, attached to FAM fluorescent label, (CATCATCAATCCCC).

STATISTICAL AND GENETIC ANALYSES

The analysis of gene and genotypic frequencies, as well as the heterozygosity for each gene in each breed, was performed according to Nei (1978) by using the BIOSYS-2 program (Swofford *et al* 1997). The number of alleles per locus (A), allele frequencies, observed heterozygosity (Ho) and expected heterozygosity (He) for genetic variation within local populations in the six porcine populations of this work have been estimated according to Hartl and Clark (1997) and Hedrick (2000).

We examined the total genetic diversity (HT), genetic diversity within populations (HS), genetic diversity among populations (DST), and genetic differentiation coefficient (GST) in genetic diversity within and among populations (Weir and Cockerham 1984, Hedrick 2000, Culley *et al* 2002).

By using Wright's F-statistics (Wright 1978), the inbreeding coefficients between individuals within a population (Fis) and genetic differentiation between populations (Fst) were calculated.

The Fis coefficient is a measure of the deviation of the panmictic frequencies, in terms of excess or deficiency of heterozygotes, caused by the tendency for crosses to occur between related individuals; it is defined as the probability that two alleles in an individual are identical by descent with respect to the subpopulation. The value of this index varies between -1 and 1. The negative values indicate an excess of heterozygotes, with respect to the Hardy-Weinberg equilibrium while positive values indicate a deficiency of heterozygotes (inbreeding). To evaluate whether the Fis values were significantly different from zero, a test $\chi^2 = F2N(k-1)$ was performed with df = [k (k-1)] / 2, where F is the inbreeding coefficient, N is the sample size and k is the number of alleles (Li and Horvitz 1953).

Wright's Fst (Wright 1978) measures the effect of genetic subdivision or the reduction of heterozygosity in a population due to genetic drift; it is defined as the correlation of alleles of different individuals in the same population and varies between zero and one. When the estimate of Fst is 0.050 or less, it does not imply that genetic differentiation is negligible (Wright 1978). The zero value indicates panmixia or random matings, all subpopulations are in equilibrium and no genetic divergence occurs in the population. The value of one indicates complete isolation, an extreme subdivision of the population. To find out if the Fst values were significantly different from zero, we calculated $\chi^2 = 2NFst(k-1)$ with df = [(k-1) (s-1)], where N is the sample size, k is the number of alleles, and s is the number of subpopulations, in this case, the number of breeds (Workman and Niswander 1970).

Gene flow and genetic distances were also calculated. Gene flow is the transfer of genetic material from one population to another (Nm) (Slatkin 1987) and genetic distances (Nei 1978) reflect the number of changes that have occurred since the separation of two populations and is appropriate when populations diverge because of drift and mutations. This measure was based on the "normalised identity", which expresses the probability that when choosing one allele at random from each of the two populations considered, these are identical (Weir 1996, Ryman and Leimar 2008). The Nei distances used were corrected for small sample sizes (Nei 1978).

RESULTS

The allelic and genotypic frequencies are indicated in table 1. For *IGF2*, only Celta, Iberian Extremeño, and Iberian Andalusian populations showed significant deviations ($P \leq 0.05$) in the Hardy-Weinberg (HW) equilibrium, which could indicate a trend of directed matings or selection

[3] Thermo Fischer Scientific, (Wilmington, DE, USA).

[4] Thermo Fischer Scientific, (Wilmington, DE, USA).

in these populations. The other populations were in HW equilibrium ($P \geq 0.05$). For *MC4R*, all populations were in HW equilibrium ($P \geq 0.05$).

In total, two alleles per locus in the two analysed loci were identified in all populations. The number of polymorphic loci was estimated, considering that a locus was polymorphic only if the frequency of the most common allele was less than an arbitrary value, which was typically 0.99. Similarly, only the Pampa Rocha, Bizaro Portuguese, and Duroc populations displayed polymorphism in the *IGF2* locus; this locus was considered non-polymorphic in the rest populations analysed. In contrast, all the analysed populations displayed polymorphism in the *MC4R* locus, except for wild boar samples that allele G was fixed.

The heterozygosity is indicated in table 2, in the *IGF2* and *MC4R* locus. The Celta, Iberian Extremeño and Iberian Andalusian populations had the lowest average genetic diversity within the populations; that is, the lowest He and Ho heterozygosities.

The genetic diversity (D) between populations is presented in table 4, being the greatest estimated distance between the populations of Celta and Duroc.

It was also calculated the coefficient of genetic differentiation for all loci, obtaining a value of GST = 0.5027, which indicated a genetic diversity of 50.27% among the populations and a genetic diversity of 49.73% within them.

WRIGHT'S F STATISTICS

The results obtained are shown in table 3. A positive Fis was obtained only in the Duroc breed, although a very low value; the rest of breeds did not indicate consanguinity,

Table 1. Allelic and genotype frequencies for the *IGF2* and *MC4R* genes analysed in the six pig breeds.

Breed	Allelic frequency		Genotype frequency		
	A	G	AA	AG	GG
		IGF2			
Pampa Rocha	0.01	0.99	0.00	0.02	0.98
Celta	0.00	1.00[a]	0.00	0.00	1.00 [a]
Bizaro Portuguese	0.02	0.98	0.00	0.04	0.96
Duroc	0.85	0.15	0.72	0.25	0.03
Iberian Extremeño	0.00	1.00 [a]	0.00	0.00	1.00 [a]
Iberian Andalusian	0.00	1.00 [a]	0.00	0.00	1.00 [a]
Wild boar	0.00	1.00 [a]	0.00	0.00	1.00 [a]
		MC4R			
Pampa Rocha	0.59	0.41	0.31	0.55	0.14
Celta	0.03	0.97	0.00	0.06	0.94
Bizaro Portuguese	0.14	0.86	0.00	0.29	0.71
Duroc	0.57	0.43	0.33	0.50	0.17
Iberian Extremeño	0.08	0.92	0.00	0.17	0.83
Iberian Andalusian	0.04	0.96	0.00	0.08	0.92
Wild boar	0.00	1.00[a]	0.00	0.00	1.00[a]

[a]For the *IGF2* gene only the Celta, Iberian Extremeño, and Iberian Andalusian population groups showed significant deviations ($P \leq 0.05$) for the Hardy-Weinberg equilibrium analysis (HW). The other populations are in equilibrium of HW ($P \geq 0.05$).
For the *MC4R* gene all populations are in HW equilibrium ($P \geq 0.05$).

Table 2. Average expected and observed heterozigosity, on the loci analysed in the six populations studied.

	He	Ho	P (0.95)[a]	P (0.99)[a]
Pampa Rocha	0.2511	0.2845	0.5000	0.5000
Celta	0.0278	0.0286	0.0000	0.5000
Bizaro Portuguese	0.1453	0.1667	0.5000	1.0000
Duroc	0.3719	0.3750	1.0000	1.0000
Iberian Extremeño	0.0764	0.0833	0.5000	0.5000
Iberian Andalusian	0.0384	0.0400	0.0000	0.5000

[a]Threshold 95%, and 99% indicates that the locus is considered polymorphic if the most frequent allele does not exceed 95% or 99%, respectively.

with negative Fis values, Fis average values for each locus was negative for both loci.

The estimated values for Fst, according to Weir and Cockerham (1984), are indicated in table 5. The values obtained suggested that most of the genetic diversity was found within the populations.

A value of Nm > 1 indicates high gene flow between the populations (Wright 1969). The values of the genetic flow among the populations are shown in table 6. The genetic distances of Nei (1978) are presented in table 7.

Table 3. Fis values for each of the six porcine populations analysed.

Population	Fis	(IC 95 %)
Pampa Rocha	–0.12433	(–0.39877 - 0.10882)
Celta	–0.01493	(–0.06250 - –0.00000)
Bizaro Portuguese	–0.13597	(–0.22401 - –0.06344)
Duroc	0.00426	(–0.24877 - 0.25072)
Iberian Extremeño	–0.06977	(–0.17949 - 0.00000)
Iberian Andalusian	–0.02128	(–0.09091 - 0.00000)

Table 4. Genetic diversity (D) between each pair of populations analysed.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha		0.64812	0.38121	0.76883	0.47787	0.57467
Celta			0.06029	1.21508	0.01429	0.01494
Bizaro Portuguese				0.99213	0.00315	0.03811
Duroc					1.03007	1.10591
Iberian Extremeño						0.00318
Iberian Andalusian						

Table 5. Fst values according to Weir and Cockerham (1984) for each pair of populations studied.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha		0.47697	0.31696	0.53645	0.37990	0.43711
Celta			0.05851	0.70331	0.01419	0
Bizaro Portuguese				0.62921	0.00314	0.03740
Duroc					0.64302	0.66909
Iberian Extremeño						0

Table 6. Gene flow Nm (Wright, 1969) between each pair of populations analysed.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha		0.27	0.54	0.22	0.41	0.32
Celta			4.02	0.11	17.37	a
Bizaro Portuguese				0.15	79.36	6.44
Duroc					0.14	0.12
Iberian Extremeño						a

[a]The gene flow between these populations has not been estimated, to reach values Fst ≤ 0, between these indicated populations.

Table 7. Genetic distances of Nei (1978) among the six porcine populations studied.

Population	Pampa Rocha	Celta	Bizaro Portuguese	Duroc	Iberian Extremeño	Iberian Andalusian
Pampa Rocha (58)	0.000	0.189	0.125	0.816	0.156	0.182
Celta (35)	0.189	0.000	0.004	1.123	0.000	0.001
Bizaro Portuguese (45)	0.125	0.004	0.000	0.989	0.000	0.003
Duroc (30)	0.816	1.123	0.989	0.000	1.080	1.114
Iberian Extremeño (24)	0.156	0.000	0.000	1.080	0.000	0.000
Iberian Andalusian (25)	0.182	0.001	0.003	1.114	0.000	0.000

DISCUSSION

During the process of selection, genetic variability can decrease compared with that of the original population. This decrease in genetic variability is a consequence of the selection process that involves the criteria and methods of selection with any degree of sophistication or use of current advanced techniques (Meffe 1986, Leberg 1992, Wellmann and Bennewitz 2019).

From the results obtained in this work, it was deduced that each analysed locus presented a different polymorphism in each porcine breed studied. In the *IGF2* locus (table 1), only the Bizaro Portuguese and Duroc populations presented interesting polymorphisms with a frequency of the G allele (0.98) much higher than the A allele (0.02) in the Bizaro Portuguese, unlike that the A allele (0.85) occurred at a higher frequency than the G allele (0.15) in Duroc; the A allele was observed in the Pampa Rocha population too but a very low frequency (0.01), similar to the frequency obtained by Burgos *et al* (2019) in this breed. In this locus, our results are very similar to those obtained by Fernandez *et al* (2017) for the Bizaro Portuguese breed (G allele with 0.99 and A allele with 0.01) and Burgos *et al* 2019 (G allele with 0.981 and A allele with 0.019). The rest of the populations do not have a frequency of A allele, as the G allele was fixed for the animals studied.

Fassa *et al* (2015) confirmed the greater effect of the A allele on BF and feed conversion, reporting that those pigs with the A allele had 1.8 mm less fat and better-feed conversion (they consumed 0.3 kg less of feed per kg of pork produced).

Oczkowicz *et al* (2009) reported that the frequency of the A allele was higher in the breeds or lines subjected to greater selection pressure for leanness and that the A allele increased the average daily weight gain and decreased consumption in Landrace populations. Fontanesi *et al* (2010, 2012) analysed a population of Large White Italian animals and, using as a registry of breeding values for BF, found that the animals with the highest expected breeding value for this character had a lower frequency of the G allele (0.38), whereas the animals with the lowest expected breeding value for BF had a higher frequency of the G allele (0.72).

In the *MC4R* locus, our results indicate that the populations of the Pampa Rocha and Duroc breeds have higher frequencies of the A allele (0.59 and 0.57, respectively), although they are values close to the G allele frequencies (0.41 and 0.43); however, their genetic structure is optimised for the objectives of higher performance, lower BF, and greater lean weight. Ovilo *et al* (2006), associated the identified missense mutation (Asp298Asn) located in a highly conserved region of this gene with backfat depth, feed intake and growth rate in different porcine lines. Our results differ slightly from those obtained by Klimenko *et al* (2014), who found a higher frequency of G allele (0.71) compared with the A allele (0.29) in other pig breeds. It should be noted that Park *et al* (2002) and Fassa *et al* (2015) did not find significant differences between *MC4R* genotypes and production characteristics, except for the wild boar samples.

Population genetics is the discipline studying the genetic diversity and structuring of populations and the processes governing them, including changes in allele frequencies over time, and further embracing the aspects of quantitative genetics (Hartl and Clark 1997, Hedrick 2000). Our main objective was to determine the genetic variability of these pig populations because the use of genetic data in the establishment of conservation units is of great importance. According to Erwin (1991), it is necessary to recover and maintain processes within the species itself, instead of conserving only distinctive phenotypes, therefore, the utility of the genetic data is magnified. This argument is based on the fact that the functioning of microevolutionary processes requires intraspecific genetic variability that, although not an innovative perspective in conservation, was already identified in the 1970s (Frankel 1974) and has been strengthened since then by evolutionary biologists (Templeton 1986).

A good measure of genetic variation is the average frequency of heterozygous individuals per locus or, similarly, the heterozygosity of the population (Fontdevila and Moya 1999). In our work, the observed heterozygosities (Ho) were higher than those expected (He) (table 2), which was an indication that the heterozygosity of all the studied breeds was good and did not show consanguinity. Indeed, Fis values were mostly negative (Fis negative values represent the absence of inbreeding or consanguinity) (table 3) except for the Duroc population which, although a positive value, was a very low value. According to Hartl (2000) positive magnitudes of the Fis, Fis is considered low if Fis = 0 - 0.05, average Fis = 0.06 - 0.15, high Fis = 0.16 - 0.25 and very high Fis > 0.25.

The values obtained in our work for genetic diversity (D) among the populations range from the lowest estimated value, corresponding to the Bizaro Portuguese and Iberian Extremeño populations (table 4), to the highest value obtained, between the Duroc and Celta. As reported by Ryman and Leimar (2008), the effect of the mutation is small on the GST value in the early stages of divergence of populations, but it is unclear how long it has been since the separation of ancestral populations and whether the mutation may have affected heterozygosity. It is possible to think that the effect of the mutation is very small on that genetic diversity and that selection has been the most important effect on diversity. It is interesting to see how the genetic diversity obtained in our populations reaches a value of GST = 0.5027, which indicated a genetic diversity of 50.27% among the populations and a genetic diversity of 49.73% within them. These are expected results since that genetic diversity will be greater between different populations and breeds than within each population;

however, these results indicate that at one time there were genetic exchanges between all of them.

Similarly, for the estimated Fst (table 5), which measure the genetic differentiation between populations, the lowest value obtained is again between the Bizaro Portuguese and Iberian Extremeño (0.00314) and the highest value between the Celta and Duroc populations (0.70331). Regarding gene flow (Nm) or exchange of genes (table 6) that has occurred in the history in these populations, the highest value (79.36) was obtained between the Bizaro Portuguese and Iberian Extremeño, therefore, when having a greater gene flow, a lower genetic distance has been obtained (table 7) between both populations (0.00). In contrast, the lowest gene flow (Nm) corresponded to the Duroc and Celtic populations (0.11), along with the greatest genetic distance between these same populations (1,123).

Genomic selection is becoming a reality in livestock species. As indicated by Samoré and Fontanesi (2016), the applications of genomic selection are opening new opportunities in pig breeding. It is expected that the complete sequencing data will provide the best prediction accuracy since all causal mutations underlying a trait can be included (Zhang *et al* 2018). But sequencing is still too expensive for implementation in a large number of animals. Therefore, low-coverage DNA sequence fragment analysis strategies reduce costs and are well accepted.

In conclusion, it is very interesting to note that in our results the Pampa Rocha and Bizaro Portuguese autochthonous breeds present a certain frequency of the beneficial A allele at the IGF2 locus, although with a much lower frequency than that of the Duroc breed.

Moreover, in the Celta, Iberian Extremeño and Iberian Andalusian populations the imbalance observed for the alleles of the *IGF2* locus can be considered, in its broadest sense, to be due to selection, that is, that there had directed crosses at some point in their history. Finally, it is very pleasing to verify that the heterozygosity in the *MC4R* locus is high in all the analysed populations, which is indicating a good genetic "health" in all of them including the autochthonous breeds, knowing that they have started from a smaller number in their population components. These results could help breeders to manage their livestock and, at the same time, they highlight the need to continue controlling their populations through genetic analyses to avoid the loss of genetic variability and the presence of inbreeding at all times.

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Austral J Vet Sci **52, 95-101 (2020)**

ORIGINAL ARTICLE

Serum biomarkers of endothelial glycocalyx injury in canine parvoviral infection

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ABSTRACT. Canine parvoviral enteritis (PVE) is one of the most common diseases in young dogs. A range of diseases and inflammatory conditions can cause endothelial glycocalyx (eGCX) disruption, therefore, this study aimed to determine the presence of eGCX damage in dogs with PVE using serum biomarkers of eGCX, and to evaluate their prognostic importance among survivor and non-survivor dogs. Twenty dogs diagnosed with PVE and 10 healthy dogs of both sexes, mixed-breed, and under 6 months of age were included in the study. Clinical examination, blood gas analysis, and complete blood cell counts of the dogs were performed. To detect the eGCX injury, serum endothelial cell-specific molecule-1 (ESM-1), syndecan-1 (SDC-1), angiopoietin-2 (Ang-2), and heparan sulfate (HS) levels were measured. Results showed that at the time of admission serum levels of ESM-1 were higher in dogs with PVE compared to that of the healthy dogs. Dogs with PVE were further assigned into two groups: survivors (n:10) and non-survivors (n:10). The ESM-1 had high sensitivity and specificity to differentiate between survivor and non-survivor dogs with values of 100% and 67%, respectively, with at an optimum cut-off point of ≥460 pg/mL. We concluded that higher levels of ESM-1 in dogs with PVE may indicate eGCX injury when compared to healthy dogs. Also, the high levels of serum ESM-1 in non-survivor dogs suggest that serum ESM-1 may carry some prognostic usefulness for predicting mortality in dogs with PVE.

Key words: vascular endothelium, dog, endothelial cell-specific molecule-1, sepsis, outcome.

INTRODUCTION

The vascular endothelium is the largest organ in the body, forming an interface between the bloodstream and the blood vessel wall. The luminal surface of all vascular endothelial cells is covered by the endothelial glycocalyx (eGCX), which consists of membrane-bound negative charged proteoglycans, glycoproteins, glycolipids, and glycosaminoglycans (Reitsma *et al* 2007). The main functions of eGCX are maintaining permeability, preventing leukocytes and thrombocyte attachments with endothelium, mechano-transduction, protecting endothelial cells, and reducing inflammatory effects (Hartawan and Wiryana 2019). Previous studies have shown that conditions such as diabetes, chronic kidney disease, hypernatremia, hypervolemia, and ischemic-reperfusion injury can also lead to the eGCX injury (Rehm *et al* 2001, Nieuwdorp *et al* 2006, Oberleithner *et al* 2011, Vlahu *et al* 2012, Padberg *et al* 2014). Furthermore, inflammatory conditions such as systemic inflammatory response syndrome and sepsis may also cause eGCX disruption (Henry and Duling 2000, Nelson *et al* 2008). Endothelial cell-specific molecule-1 (ESM-1) or endocan, syndecan-1 (SDC-1), angiopoietin-2 (Ang-2), and heparan sulfate (HS) are the most investigated metabolites of eGCX shedding in critically ill patients (Ioakeimidou *et al* 2017, Uchimido *et al* 2019).

Canine parvoviral enteritis (PVE) is one of the most important infectious causes of mortality in young puppies (Carmichael 2005). The early lesions consist of necrosis of the crypt epithelial cells in the enteric form of the disease. The villi and lamina propria may collapse completely as a result of the loss of crypt epithelium and the failure of replacing sloughed villous epithelial cells. The loss of digestive epithelium and absorptive surface area presumably results in diarrhoea caused by the combined effect of maldigestion and malabsorption. Death may follow as a result of dehydration, electrolyte imbalance, endotoxic shock, or secondary septicemia (Nandi and Kumar 2010).

Season, purebred nature, bodyweight, vomiting, leukopenia, lymphopenia, thrombocytopenia, hypercoagulability, hypercortisolemia, hypothyroxinemia, hypoalbuminemia, elevated C-reactive protein and tumour necrosis factor, hypocholesterolemia, and hypocitrullinemia have been the preferred variables as diagnostic and prognostic biomarkers in PVE (Schoeman *et al* 2013). However, there is no literature information about the usage of eGCX in dogs with PVE. Therefore, the present study aimed to determine the presence and magnitude of eGCX damage in dogs with PVE using serum biomarkers of eGCX and to evaluate their prognostic importance to distinguish between the survivor and non-survivor dogs.

Received: 07.04.2020.

Accepted: 24.07.2020.

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MATERIAL AND METHODS

ANIMALS

The study protocol was approved by the ethics committee of the Faculty of Veterinary Medicine, Selcuk University (permit number: 2018/140). Twenty dogs suffering from PVE and 10 healthy dogs of both sexes, mixed-breed, and under 6 months of age were included in the study during the years 2018-2019.

All the dogs with PVE had clinical observations of vomiting, anorexia, lethargy, and diarrhoea ranging from mucoid to bloody. Some of the dogs already had sepsis symptoms such as hypothermia, tachycardia, hyperpnea, and severe dehydration at the time of admission. The diagnosis of PVE was confirmed by faecal CPV Ag test (Asan Easy Test PARVO, Asan Pharma. CO. LTD., Gyeonggi-do Korea, sensitivity: 99.5%, specificity: 99.8%). None of the dogs enrolled had been vaccinated before the beginning of the study. All the dogs in the PVE group received the same treatment including fluid therapy, antibiotics, and other supportive treatments (antiemetics, vitamin, and amino acid supplementation) during the hospitalisation period. The healthy dogs were chosen among puppies brought to our clinics for routine vaccination and were assigned to the healthy group according to physical examinations, laboratory findings, and negative faecal CPV Ag test results.

SAMPLE COLLECTION

Five mL of blood were collected by jugular venipuncture at the time of admission and before discharge from the hospital. One mL of the collected sample was anaerobically transferred into sodium heparin containing plastic syringes and blood gas analysis was performed immediately. Syringes were prepared by aspirating a small volume of liquid heparin (5000 IU/ml) (Nevparin®, Mustafa Nevzat, Turkey) and then expelling it. The heparin layer covering the inside of the syringe prevents the blood sample from coagulation. An extra mL of the blood was put into the tubes containing K_3EDTA and a complete blood count (CBC) analysis was performed immediately. The remaining 3 mL of collected blood was put into the tubes without anticoagulant, centrifuged at 2000 x *g* for 5 min at 4 °C, and serum samples were extracted. They were stored at -80 °C and defrosted immediately before the enzyme-linked immunosorbent assay (ELISA) analysis.

BLOOD GASES AND COMPLETE BLOOD COUNT

A venous blood gas analysis which included pH, the partial pressure of carbon dioxide (pCO_2), lactate, sodium (Na), calcium (Ca), chloride (Cl), potassium (K), glucose, base excess (BE), and bicarbonate (HCO_3) was performed using an automatic blood gas analyser (ABL 90 Flex, Radiometer, USA). CBCs including total leukocytes, lymphocytes, granulocytes, erythrocytes, mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), haemoglobin, and platelets were done using an automatic cell counter (MS4e, Melet Schlosing Laboratories, France).

ENDOTHELIAL GLYCOCALYX RELATED BIOMARKERS

Serum endothelial cell-specific molecule-1 (ESM-1), syndecan-1 (SDC-1), angiopoietin-2 (Ang-2), and heparan sulfate (HS) levels were measured according to the manufacturer's protocol using canine ESM-1 commercial sandwich ELISA kit (MyBioSource, USA, Lot: 20190809C), canine syndecan-1 commercial ELISA kit (MyBioSource, USA, Lot: 08/2019), canine angiopoietin-2 commercial ELISA kit (MyBioSource, USA, Lot: 36343442), and canine heparan sulfate commercial ELISA kit (MyBioSource, USA, 20190809C). Intra-assay coefficients, inter-assay coefficients, and minimum detectable concentrations were < 10%, < 10% and 1 pg/mL for ESM-1, < 15%, < 15% and 6.25 µg/mL for SDC–1, ≤ 8%, ≤ 12% and 0.15 ng/mL for Ang-2, < 10%, < 10% and 0.1 ng/mL for HS.

STATISTICAL ANALYSIS

Data analysis was performed using statistical software (SPSS 25.00). To determine whether the variables had normal distributions, the one-sample Kolmogorov-Smirnov test was used. Parametric data were evaluated by Student t-test while mean ± standard deviation (SD) and non-parametric data were evaluated by Mann-Whitney U test as median (min/max). A linear regression analysis was made to determine the independent predictors of mortality. The prognostic values of endothelial related biomarkers were evaluated using receiver operating characteristic (ROC) curve analysis to determine the prognostic cut-off values for the best differentiation between survivors and non-survivors of PVE. Probability curve (survival) was also conducted for serum ESM-1. Statistical significance was considered as $P<0.05$.

RESULTS

The dogs with PVE presented various weights (8.92±4.81 kg) and ages (3.8±1.28 months) and the healthy dogs had similar weights (10±1.95 kg) and ages (3.2±0.48 months). There were no statistically significant differences in body weight and age between the groups ($P>0.05$). Ten dogs in the PVE group survived and were discharged from the hospital 72 hours after the admission and were followed up for the next 14 days by a telephone call to the owners. During the hospitalisation period, 9 dogs in the PVE group died within the first 24 hours and another dog died between 24 and 48 hours. None of the dogs was euthanised during the study.

Clinical findings showed that dogs with PVE had a higher respiration rate and heart rate (47.09±18.9 and 143.53±33.65, respectively) than healthy dogs (22.13±6.25 and 135.60±6.96, respectively) (P<0.05). The body temperature did not differ between PVE (38.05±1.66) and healthy dogs (38.47±0.42) (P>0.05). The results also revealed that at the time of admission the survivor dogs were not as severely ill as the non-survivor dogs. Higher respiration rate, heart rate, dehydration rate, prolonged capillary refill time (CRT), more variations in consciousness, and haemorrhagic diarrhoea were the most characteristic features in the survivor and non-survivor dogs (table 1).

The blood gases analysis and CBC results showed no significant differences between healthy dogs and dogs with PVE (P>0.05). Also, there were no significant differences between the time of admission and discharge from the hospital (supplementary table 1[1]).

The results of serum biomarkers of endothelial glycocalyx showed that levels of ESM-1 were higher in dogs with PVE compared to those of healthy dogs and before discharge (table 2, P<0.05). There were no significant changes in the levels of SDC-1, Ang-2, and HS between the healthy dogs and dogs with PVE (table 2, P>0.05).

The linear regression analysis showed a significant slope (F (1,18) = 7.711, P<0.012) with an R^2 = 0.300 to predict mortality for dogs with PVE based on their serum ESM-1 levels (table 3, supplementary figure 1[2]). The same slope was not found for serum levels of SDC-1, Ang-2, and HS (P>0.05). Also, the results of the PVE group showed that the concentrations of ESM-1 from the non-survivor dogs were significantly higher than those of the survivor dogs (P<0.05) (supplementary figure 2[3]). Receiver operating characteristic curve (ROC) analysis for the utility of ESM-1, SDC-1, Ang-2 and HS in differentiating between the survivor and non-survivor dogs estimated an area under the curve (AUC) of 0.821 (p= 0.022, 95% CI=0.615-1.000) for ESM-1 (table 4, figure 1). An optimum cut off point of 460 pg/mL corresponds to a sensitivity of 100% and a specificity of 67% for prediction of mortality (table 4). Survival probability curve for serum endothelial cell-specific molecule-1 (ESM-1) concentrations in dogs with PVE demonstrated that the highest serum concentrations of ESM-1 were associated with the lower prediction of survival in dogs with PVE (figure 2).

DISCUSSION

Acute enteritis is the most common clinical manifestation of PVE mostly seen in puppies up to 6 months of age. The non-specific clinical signs in the early stages of the disease include depression, anorexia, and lethargy. Vomiting and mucoid to hemorrhagic diarrhoea are the typical signs of the PVE and they develop in the later stages of the disease (Goddard and Leisewitz 2010). Likewise, the puppies in the PVE group of this study had variable signs of anorexia and depression. Also, all of the dogs assigned

Table 1. Clinical parameters in the healthy (n:10), the survivor (n:10) and the non-survivors (n:10) dogs. Body temperature, respiration rate, and heart rate are presented as mean± SD. Dehydration rate, capillary refill time, mucosal membranes, mental status, and the type of diarrhea are presented as median and range in parentheses.

Parameter	Healthy dogs	Dogs with PVE at time of admission	
		Survivors	Non-survivors
Body temperature (°C)	38.47±0.42	38.65±0.38	38.21±0.71
Respiration rate (bpm)	22.13±6.25	26.22±5.39 [a,b]	37.12±3.28[a]
Heart rate (bpm)	135.60±6.96	141.11±18.12 [a,b]	158.36±10.29 [a]
Dehydration rate (%)	1 (1)	2 (1-2) [b]	3 (1-3) [a]
Capillary refill time (sec.)	1 (1)	2 (1-2) [a,b]	3 (2-3) [a]
Mucous membranes	2 (2)	3 (1-4) [a]	3 (1-4) [a]
Mental status	4 (4)	3 (1-4) [a,b]	2 (1-3) [a]
Type of diarrhea	1 (1)	2 (2-3) [a,b]	3 (2-3) [a]

[a]Comparison between healthy dogs (P<0.05).
[b]Comparison between non-survivor dogs (P<0.05).
Dehydration rate: <5% (1); 6-8% (2); 9-10% (3); >10% (4).
Capillary refill time: <2 sec (1); 3-4 sec (2); 4-5 sec (3).
Mucous membranes: hyperemic (1), normal (2), slightly pale (3), pale (4).
Mental state: comatose (1), depressive (2), alert (3), active (4).
Diarrhoea: none (1), mucoid diarrhoea (2), hemorrhagic diarrhoea (3).

1 Available at www.australjvs.cl/ajvs

2 Available at www.australjvs.cl/ajvs

3 Available at www.australjvs.cl/ajvs

Table 2. Comparison of eGCX related biomarkers in the healthy dogs (n:10), dogs with PVE at admission (n:20), and before discharge (n:10). Data are presented as mean ± SD and median and range in parentheses.

Parameter	Healthy dogs	Dogs with PVE	
		Admission	Before discharge
ESM-1 (pg/mL)	283.64±114.15	644.11±340.36 [a]	313.26±82.60 [b]
SDC-1 (µg/mL)	25 (12-63)	28 (13-168)	35 (14-114)
Ang-2 (ng/mL)	9.01±5.67	8.97±7.02	9.80±3.51
HS (ng/mL)	5.78±3.19	7.35±2.21	8.09±2.72

[a]Comparison between healthy dogs (P<0.05).
[b]Comparison between time of admission (P<0.05).
ESM-1, endothelial cell-specific molecule-1; SDC-1, syndecan-1; Ang-2, angiopoetin-2; HS, heparan sulfate.

Table 3. Summary of regression analysis of serum biomarkers of eCGX in 10 survivors and 10 non-survivor dogs with PVE at the time of admission. Data are presented as mean ± SD.

Parameter	Dogs with PVE		
	Survivors	Non-survivors	P-value
ESM-1 (pg/mL)	313.26±82.60	580.48±292.87	0.012
SDC-1 (µg/mL)	50.04±36.14	47.68±46.66	0.901
Ang-2 (ng/mL)	9.80±3.51	7.63±3.34	0.199
HS (ng/mL)	8.09±2.07	6.13±1.48	0.056

ESM-1, endothelial cell-specific molecule-1; SDC-1, syndecan-1; Ang-2, angiopoetin-2; HS, heparan sulfate.

to the PVE group had hemorrhagic diarrhoea either at the time of admission or later during the hospitalisation period.

Previous studies (Otto *et al* 1997, de Laforcade *et al* 2003, Prittie 2004, Yilmaz and Senturk 2007) have demonstrated that disruption of the intestinal barrier due to canine parvovirus infection can lead to the translocation of bacteria and endotoxins released from the intestinal tract into the peripheral circulation, with the subsequent development of coliform septicemia. This condition may then lead to SIRS and sepsis. Total leukocyte count (<6×10^9/L or >16×10^9/L), heart rate (>160 bpm), respiratory rate (>25 breaths/min), and body temperature (<37 °C or >39.4 °C) are the described criteria for the definition of SIRS and sepsis in dogs (Silverstein and Hoper 2015, Ok *et al* 2015). These findings were remarkable in the dogs with PVE. The rapid deterioration of the clinical appearance in the non-survivor dogs may be related to the development of SIRS and sepsis.

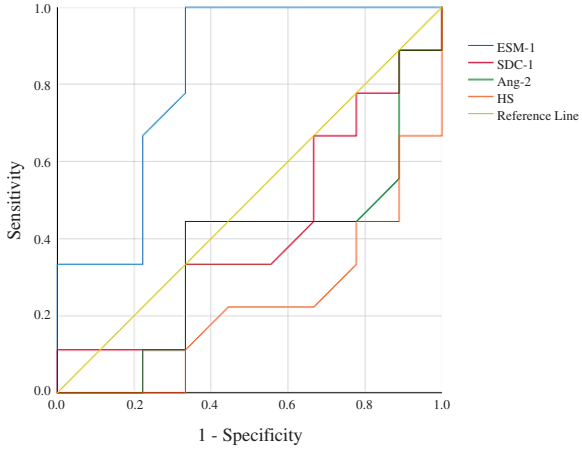

Figure 1. Receiver operating characteristic curve analysis for the differentiation between the survivor and non-survivor dogs with parvoviral enteritis based on the serum endothelial cell-specific molecule-1 (ESM-1), syndecan-1 (SDC-1), angiopoetin-2 (Ang-2) and heparan sulfate (HS) concentration at the time of admission. Results showed that serum ESM-1 (blue line) is the more sensitive and specific biomarker for predicting mortality in the dogs with CPV.

Studies reporting the mortality rates in the canine PVE have shown that it varies between 25% and 35%, while it can reach 91% without treatment (Ling *et al* 2012). Kocaturk *et al* (2010) reported a higher mortality rate (53.4%) and shorter survival length in the cases of canine PVE. They concluded that insufficient immune system development in younger puppies, sepsis, and related complications were the main causes of death in their study. Our findings indicated that at the time of admission, symptoms of SIRS like tachypnea, tachycardia, hypovolemia, prolonged CRT, and mental status were more serious in the non-survivor dogs compared to the survivors. Also, anamnesis revealed that in the case of non-survivors there were approximately 48 hours between the appearance of the first symptoms (loss of appetite, vomiting, diarrhoea, and stuporous state) and admission to the hospital. As mentioned by Mylonakis

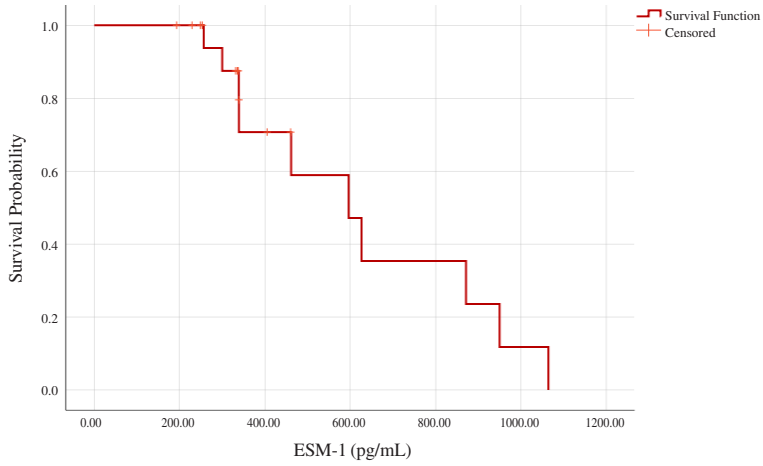

Figure 2. Survival probability curve for serum endothelial cell-specific molecule-1 (ESM-1) concentration in dogs with parvoviral enteritis suggests that the highest serum concentrations of ESM-1 are associated with a lower probability of survival in dogs with PVE.

Table 4. The area under the curve (AUC), standard error, confidence interval (95%), optimum cut-off values of ESM-1, SDC-1, Ang-2, HS, and respective sensitivity and specificity of mortality prediction in dogs with PVE.

Variable	AUC	Standard Error	P value	Asymptotic 95% Confidence Interval		Cut-off Point	Sensitivity	Specificity
				Lower Band	Upper Bound			
ESM-1	0.821	0.105	0.022	0.615	1.000	460.92	100	67
SDC-1	0.414	0.139	0.536	0.140	0.687	41.27	66	34
Ang-2	0.364	0.137	0.331	0.095	0.634	7.14	44	67
HS	0.222	0.111	0.067	0.004	0.440	7.91	44	23

ESM-1, endothelial cell-specific molecule-1; SDC-1, syndecan-1; Ang-2, angiopoetin-2; HS, heparan sulfate.

et al (2016), complications such as severe dehydration, hypoperfusion, and the delay in initiation of the treatment probably influenced the survival rates in our study.

Studies in humans with critically ill patients have reported that deterioration of the endothelium of the blood vessels is one of the major complications during systemic inflammation (Cox *et al* 2015). The vascular endothelium is the largest organ in the body and the endothelial cells line as a single layer along the inner portion of the heart, blood vessels, and lymphatic vessels (Karamysheva 2008). The glycocalyx is a gel-like layer lining the luminal surface of endothelial cells, composed of membrane-bound proteoglycans, glycoproteins, glycosaminoglycans, and adherent plasma proteins (Weinbaum *et al* 2007).

The eGCX damage can be assessed by different methods. The most common methods to measure eGCX levels in plasma and urine samples in humans are anti-thrombogenic activity assay, electron microscopy, intravital microscopy, side-stream dark-field imaging, and enzyme immunoassay. (Palud *et al* 2015, Ioakeimidou *et al* 2017, Iba and Levy 2019). Since there is no available literature data about any standardised methods for eGCX evaluation in dogs, the enzyme immunoassay method (commercial canine ELISA kits) was used in the present study to determine glycocalyx injury in dogs with PVE.

Our results showed that among the serum biomarkers used in the study, concentrations of ESM-1 were higher in dogs with PVE compared to those in the healthy dogs and time before discharge. Other serum biomarkers of eGCX did not show any significant changes between healthy dogs and dogs with PVE. The ESM-1 or endocan is a proteoglycan that is secreted by endothelial cells and the main source of synthesis is the lung endothelial cells (Béchard *et al* 2001, Seo *et al* 2015). Experimental studies of human endotoxemia demonstrated that the levels of endocan increased after lipopolysaccharide (LPS) infusion at the beginning of the inflammatory phase and returned to the baseline at 8 h (Cox *et al* 2015). A prospective study in septic patients showed that circulating levels of endocan in the septic group were significantly higher than healthy donors and patients with SIRS. They indicated that the concentration of endocan in patients with septic shock

was prominently higher than that of patients with sepsis and severe sepsis (Scherpereel *et al* 2006).

Previous studies investigating sepsis demonstrated that pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) as well as bacterial endotoxin and LPS induced the synthesis and release of endocan by endothelial cells. Finally, the sustained hypersecretion of endocan stimulated by LPS and pro-inflammatory cytokines may be in accordance with the high levels of serum endocan in patients with worse outcome (Lassalle *et al* 1996, Bechard *et al* 2000). This information suggests that the endocan release could be partly due to endothelial cell injury. Therefore, higher levels of ESM-1 in dogs with PVE compared to healthy dogs migth be related to endothelial cell injury and degradation of eGCX caused by bacterial LPS, endotoxemia, and the release of inflammatory cytokines and endothelial components into the circulation.

Our results showed a significant decrease of ESM-1 levels in the blood samples taken at the time of discharge compared to the levels at the time of admission. In human neonatal septicemia, Zonda *et al* (2019) found the level of endocan at the time of admission was significantly higher in septic neonates than in non-septic ones and remained high until the 3rd day from admission, before returning to their normal values on day 7 (Lassalle *et al* 1996, Bechard *et al* 2000, Zonda *et al* 2019). The findings of the present study suggest that dogs with PVE had high levels of ESM-1 at the time of admission and after the treatment and the resolution of the inflammatory state, while the levels of ESM-1 in the recovered dogs tended to decrease and were comparable with the healthy group.

The levels of ESM-1 were higher in non-survivor dogs. The analysis of the area under the curve also confirmed that ESM-1 levels at the cut-off point of 460 pg/mL at the time of admission had prognostic importance for discrimination between the survivor and non-survivor dogs. Scherpereel *et al* (2006) showed that endocan levels in intensive care unit (ICU) patients were higher in non-survivors than in patients who were still alive 10 days later. Their results suggested that the endocan levels in the blood of septic patients were related to the severity of illness (i.e. higher in septic shock patients when compared to patients with severe sepsis and sepsis). Another study performed by Mihajlovic *et al* (2014) showed that the concentration of endocan was higher in patients with severe sepsis-induced multi-organ dysfunction in the first 48 h. In their opinion, endothelial damage, which occurs in sepsis, may be a significant factor in the pathogenesis of sepsis-induced organ failure and death. Also, Pauly *et al* (2016) evaluated the diagnostic and prognostic value of ESM-1 in patients suffering from severe sepsis and septic shock, and found that endocan was able to predict both short- and long-term mortality which was already initiated within the first 24 h of ICU presentation. Based on our findings, ESM-1 levels were the only prognostic marker of mortality in cases of dogs with PVE. The increased levels of ESM-1 in the non-survivor dogs within the first 24 h after admission (9/10 of whole mortality) were due to the severity of clinical findings and the development of sepsis in these dogs. The AUC of 0.821, a sensitivity of 100%, and a specificity of 67% suggest that the ESM-1 may carry some prognostic usefulness for predicting mortality in dogs with PVE.

The present study had the following limitations: 1) the study population was relatively small and a re-evaluation of the hypothesis of eGCX injury in larger sample populations is needed, 2) a lack of more frequent blood measurements especially at 24th and 48th hours 3) a histopathological examination was not performed in the non-survivor dogs, 4) since the markers of organ dysfunction were not evaluated in the present study, we could not make an association between eGCX injury and organ dysfunction, especially in the non-survivor dogs. As a conclusion, we observed that the levels of ESM-1 were higher in dogs with PVE when compared to healthy dogs. Also, the concentrations of ESM-1 were higher in the non-survivors dogs than in the survivors. At the time of admission, the cut-off point of ≥460 pg/mL for ESM-1 with sensitivity and specificity of 100% and 67%, respectively, had prognostic importance for discrimination between the survivor and non-survivor dogs with canine PVE. Endothelial specific molecule-1 (ESM-1) is considered to be a promising prognostic biomarker in dogs with PVE.

ACKNOWLEDGEMENTS

This study was financially supported by the Selcuk University Scientific Research Office (Project no: 19401010).

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

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Austral J Vet Sci **52, 103-107 (2020)**

SHORT COMMUNICATION

Estimation of genetic parameters for milk yield using a random regression test-day model in first parity dairy cows under pasture-based systems of Los Ríos region in Chile

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ABSTRACT. In dairy cattle, genetic selection for milk yield was generally based on 305 days lactation records that were calculated from available monthly test-day milk yield records. A test-day milk yield record, multiplied by the number of days between the current and following test-day, was the monthly milk yield and summed to all other monthly milk yields represented a 305 days lactation yield. Cows that for any reason did not complete their lactation got a 305 days yield via correction factors assuming a common lactation curve. Random regression models allow individual deviation from a common curve. The objective of this study was to estimate genetic parameters for daily milk yield using a random regression model (RRM) in Chilean dairy cows. A data set containing 97,683 monthly test-day records of 10,528 cows from 15 commercial dairy herds of Los Ríos Region in southern Chile was used. Days in milk (DIM) were modelled using the fourth-order Legendre polynomials and the model also included, as fixed effects, contemporary group and cow age at test-day as a covariate. The average daily milk yield was 17.83 ± 5.25 kg. Average estimated heritability and repeatability from five to 305 DIM was 0.26 ± 0.02 and 0.61 ± 0.04, respectively. The heritability estimate varied from 0.23 to 0.31. Both parameters did not vary dramatically except after 270 DIM when repeatability increased while heritability decreased. Although the estimated genetic parameters did not seriously depart from the most recent results available in the Chilean literature, they are mathematically more precise for estimating the true parameters than those calculated using adjustment factors, suggesting that the model used could be the starting point to develop a genetic evaluation system for dairy cattle in Chile.

Key words: milk, test day, random regression, genetic parameters.

INTRODUCTION

Test-day models for the genetic evaluation of dairy yield traits were first used in Canada by Ptak and Schaeffer (1993) and in the case of somatic cells, they were used by Reents *et al* (1994). The task was to model test-day milk yields as a function of a known day in milk (DIM) fixed lactation curve. This procedure allowed a more accurate estimation of environmental effects by accounting for their influence in a particular DIM. Hence, the estimated breeding value of an animal in lactation includes information from all available test-day records and the estimation accuracy is therefore improved (Ptak and Schaeffer 1993). Another advantage of test-day models is that uncompleted lactation test-day records can also be used in the analyses without needing adjustment factors (Jensen 2001). However, test-day models using fixed parameters of DIM (lactation curve) assume that all cows follow the same shape of the chosen lactation curve, these do not allow for random individual cow deviations from the fixed lactation curve used in a particular model (Jamrozik and Schaeffer 1997). Test-day models using random regressors allow for individual cow departure from a common fixed lactation curve, they were first implemented by Jamrozik and Schaeffer (1997) on a commercial basis for Canadian Holstein. The basic principle of random regression models applied to dairy cattle consists of fitting common to all animals' average lactation curves and specific curves describing individual random deviations from the average curve (Bohmanova *et al* 2008). Uribe (2001) provided a basic introduction of RRMs applied to milk yield test-day records. Schaeffer (2004) thoroughly documented the theoretical aspects, application, and structure of RRMs in animal breeding including test-day records.

Orthogonal polynomials (Kirkpatrick *et al* 1990) are widely used in RRM because they are easy to fit and the correlation among parameters has been proven low, albeit it has no biological meaning (Schaeffer 2004, Pool and Meuwissen 1999). The use of orthogonal polynomials to model lactation curves in genetic evaluations of dairy cattle has been implemented in New Zealand (Harris *et al* 2007), Germany, Canada, and the United Kingdom (Strabel *et al* 2005). On the other hand, Chile has not implemented a centralized dairy cattle genetic improvement program like other countries.

Some attempts have been made to estimate the genetic parameters of the Chilean Holstein population but none of them has used RRMs (Elzo *et al* 2004, Montaldo *et al* 2017, Uribe *et al* 2017). The objective of this study was to estimate genetic parameters such as heritability (h^2) and repeatability (*rep*) for daily milk yield, using orthogonal polynomials in a random regression test-day model in cows of Los Ríos Region, Chile.

Received: 09.04.2020.

Accepted: 24.06.2020.

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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 pedigree files, 2,350 ancestors without records were included. Data were gathered from 1996 to 2019 in 15 commercial dairy herds of Los Ríos Region, 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.

Calving age was available in the data set, therefore, 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 milk were deleted as well as records below six and above 305 DIM.

To account for DIM monthly milk yield test-day records were modelled using the fourth-order orthogonal polynomials, as described by Kirkpatrick *et al* (1990). Let y_t be the dependent variable kg of milk measured on day t of lactation, the polynomial equation can be written as:

$$y_t = b_0P_0 + b_1P_1 + \ldots + b_nP_n \qquad Eq.\ 1$$

Where b_i are the estimated regression coefficients and P_i is standardized to the unit of time orthogonal polynomial (Schaeffer 2004). Following Schaeffer (2004) the standardized trajectory (x_t) chosen in this study, from six to 305 DIM, can be expressed from –1 (six days) to +1 (305 days) as follows:

$$x_t = -1 + 2\left(\frac{t - t_{min}}{t_{max} - t_{min}}\right) \qquad Eq.\ 2$$

Where: t is the DIM of a given test-day record, t_{min} and t_{max} are six and 335 DIM, respectively. Thus, the standardized fourth-order plus the intercept orthogonal polynomials for a daily milk yield observation can be obtained as follows (Spiegel 1971): $L_0(t) = 1$, $L_1(t) = x_t$, $L_2(t) = \frac{1}{2}(3x_t^2 - 1)$, $L_3(t) = \frac{1}{2}(5x_t^3 - 3x_t)$, $L_4(t) = \frac{1}{8}(35x_t^4 - 30x_t^2 + 15)$.

The random regression animal model was:

$$y_{ijkl} = C_i + b_{ijkl}(Age) + \sum_{h=0}^{4} \beta_{jh} L_{ijkl} + \\ + \sum_{h=0}^{4} \alpha_{kh} L_{ijkl} + \sum_{h=0}^{4} \lambda_{kh} L_{ijkl} + e_{ijkl} \qquad Eq.\ 3$$

Where: y_{ijkl} = record l of cow k of herd j in contemporary group i. C_i fixed effect of the contemporary group i. b_{ijkl} fixed regression coefficient of daily milk yield on the age of the cow (Age). β_{jh} = fixed regression coefficients within-herd j. α_{kh} = genetic random regression coefficients for animal k. λ_{kh} permanent environmental random regression coefficients for animal k. e_{ijkl} = random residual effect associated with y_{ijkl}.

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

In matrix notation the model can be written as:

$$y = Xb + Za + Wp + e \qquad Eq.\ 4$$

Where vector b includes C_i, b_{ijkl} and β_{jh}, vector a includes five random regression coefficients per each animal included in the analysis, p is a vector of five random permanent environmental coefficients per each cow with records, and e is a vector of heterogeneous residual effects. Matrices X, Z and W are the incidence and covariate matrices. The (co)variance structure of the model was:

$$\begin{pmatrix} a \\ p \\ e \end{pmatrix} \sim N(0, V) \qquad Eq.\ 5$$

With

$$V = \begin{pmatrix} G \otimes A & 0 & 0 \\ 0 & P \otimes I & 0 \\ 0 & 0 & R \end{pmatrix} \qquad Eq.\ 6$$

The genetic covariance matrix of the five random regression coefficients is G, with elements denoted by g_{ij} for i and j going from 0 to 4. A is the additive genetic relationship matrix and $\otimes$ is the Kronecker product function. P is a 5x5 permanent environmental covariance matrix with elements denoted by p_{ij}, I is an identity matrix of order equal to the number of cows with records. R is a diagonal matrix with elements that depend on DIM, let $R = diag(\sigma_{e_i}^2)$, following Jaffrezic *et al* (2000) and Tsuruta *et al* (2004) the i^{th} diagonal element that assumes the heterogeneous structure across DIM of R was estimated as:

$$ln\sigma_e^2 = L'c = (L_0 \quad L_1 \quad L_2 \quad L_3 \quad L_4)\begin{pmatrix} c_0 \\ c_1 \\ c_2 \\ c_3 \\ c_4 \end{pmatrix} \qquad Eq.\ 7$$

Where L is a vector of orthogonal polynomials for a given DIM and c_i is the natural log of the estimated residual coefficients.

Estimates of genetic and permanent environmental variances at DIM i were obtained as:

$$\sigma_{a_i}^2 = L_i'\hat{G}L_i \qquad Eq.\ 8$$

$$\sigma_{p_i}^2 = L_i'\hat{P}L_i \qquad Eq.\ 9$$

Where $\hat{G}$ and $\hat{P}$ are the estimated covariance matrixes for the random regression coefficients and permanent environmental elements, respectively, and L_i is a vector of orthogonal polynomials affecting DIM *i*. Likewise, the genetic covariance between any two DIM ($\sigma_{a_{i.j}}$) were estimated as:

$$\sigma_{a_{i.j}} = L_i' \hat{G} L_j \qquad \textit{Eq.}\ 10$$

Where L_j is a vector of orthogonal polynomials affecting the j^{th} DIM.

Genetic correlations, for daily milk yield between the i^{th} and the j^{th} DIM ($r_{a_{i.j}}$) were estimated as:

$$r_{a_{i.j}} = \sigma_{a_{i.j}} \Big/ \sqrt{\sigma_{a_i}^2} * \sqrt{\sigma_{a_j}^2} \qquad \textit{Eq.}\ 11$$

h^2 and *rep* at DIM *i* were calculated as:

$$h_i^2 = \sigma_{a_i}^2 \Big/ \sigma_{a_i}^2 + \sigma_{p_i}^2 + \sigma_{e_i}^2 \qquad \textit{Eq.}\ 12$$

$$rep_i = \sigma_{a_i}^2 + \sigma_{p_i}^2 \Big/ \sigma_{a_i}^2 + \sigma_{p_i}^2 + \sigma_{e_i}^2 \qquad \textit{Eq.}\ 13$$

To solve the linear model and estimate variance components the data were processed using the AIREML software[1]. For the final estimates, a value lower than 10^{-10} of the squared differences between two consecutive solution estimates was defined as the convergence criterion.

RESULTS AND DISCUSSION

The average DIM for all test-day records was 152.50 ± 84.19 days while the same parameter, considering only the last test-day of each cow, was 279.19 ± 22.36 days. The shortest lactation included in the study lasted 73 DIM, test-day records per cow ranged from one to 11 and the average was 9.28 ± 1.08.

Table 1 presents average, minimum and maximum estimated values across 300 days in milk for h^2, *rep* and genetic, permanent environmental, and residual variances. Compared with Cobucci *et al* (2005) the pattern across DIM for genetic variances was similar except that in the latter the genetic variance steadily increased from 180 DIM toward the end of the lactation. However, Gebreyohannes *et al* (2016) and Bohmanova *et al* (2008) indicated that genetic variance consistently increased from the beginning to the end of lactation, the increment changed from 0.14 toward 1.0 $(\ln(kg/day)^2$ for 30 to 300 DIM, respectively.

The estimate of h^2 across DIM reached its peak at 270 DIM (0.31) and fell to 0.24 at 305 DIM (table 2).

Table 1. Average estimate, standard deviation (SD) and minimum (Min) and maximum (Max) values throughout 300 days in milk for heritability (h^2), repeatability (*rep*), genetic (σ_a^2), permanent environmental (σ_p^2) and residual (σ_e^2) variances.

Parameter	Average	SD	Min	Max
h^2	0.26	0.02	0.23	0.31
rep	0.61	0.04	0.58	0.78
σ_a^{2*}	3.89	0.33	3.34	4.95
σ_p^{2*}	5.14	0.80	4.42	8.73
σ_e^{2*}	5.85	0.79	3.58	7.20

* = kg^2.

Table 2. Additive genetic (σ_a^2), permanent environmental (σ_p^2) and residual variances (σ_e^2), heritability (h^2), repeatability (*t*), at selected days in milk (DIM), genetic correlation ($r_{a_{i.j}}$) between the sixth and the other selected DIM, and the corresponding average daily milk yield (AMY).

DIM	σ_a^{2*}	σ_p^{2*}	σ_e^{2*}	h^2	*t*	$r_{a_{i.j}}^{**}$	AMY (kg)
6	4.95	8.14	7.20	0.24	0.65	1	16.44
30	3,83	5.79	7.07	0.23	0.58	0.85	19.79
60	4.10	5.63	6.64	0.25	0.59	0.62	20.30
90	4.09	5.09	6.17	0.27	0.60	0.53	19.41
120	3.80	4.71	5.86	0.26	0.59	0.50	18.72
150	3.48	4.61	5.72	0.25	0.59	0.50	18.01
180	3.34	4.48	5.72	0.25	0.58	0.49	16.96
210	3.57	4.43	5.74	0.26	0.58	0.45	16.69
240	4.13	4.67	5.58	0.29	0.61	0.39	16.19
270	4.39	4.93	5.00	0.31	0.65	0.37	15.61
305	3.80	8.73	3.58	0.24	0.78	0.46	15.40

* = kg^2., ** = genetic correlation between the sixth and the corresponding (*j*), in the first column, day in milk.

[1] Misztal I, Tsuruta S, Lourenco DAL, Masuda Y, Aguilar I, *et al*. 2018. Manual for BLUPF90 family programs. University of Georgia. http://nce.ads.uga.edu/wiki/doku.php?id=documentation

The estimates for heritability of Gebreyohannes *et al* (2016) varied from 0.17 and 0.42 for the first and the last test-day, respectively, and were similar to the h^2 estimates reported in this study only for the first 50 DIM, and the authors partially attributed their high h^2 estimate due to the degree of variability of the multi-breed population used in their research. Similarly, the h^2 estimates of Cobuci *et al* (2005) varied from 0.15 to 0.31 and agreed with the results presented in this study. After ignoring the estimates of the first week of lactation, the h^2 estimates of Bignardi *et al* (2011) varied from 0.20 to 0.35, their highest estimates were obtained between 196 and 273 DIM and their results are similar to those presented in this study, particularly the pattern followed by the h^2 across DIM.

The average h^2 estimate in this study was 0.26 (table 1) which is lower than the estimates reported by Elzo *et al* (2004) and Montaldo *et al* (2017) (0.29-0.34), but greater than that published by Uribe *et al* (2017) (0.16) for milk yield. The estimates of h^2 reported in the present study are important because variance components calculated using a RRM are mathematically more precise for estimating the true parameters than those calculated using adjustment factors (Schaeffer 2004), which is the case of those previously published in Chile. The expected genetic change (ΔG) using estimates of a RRM, based in the formula proposed by Rendel and Robertson (1950) $(\Delta G = i \times r_{A,\hat{A}} \times \sigma_g)$, should be higher than the genetic progress based on Elzo *et al* (2004), Montaldo *et al* (2017), and Uribe *et al* (2017) because of their lower breeding value estimation accuracy $(r_{A,\hat{A}})$.

Repeatability estimates across DIM fluctuate between 0.58 and 0.78 (table 1), the pattern across DIM is very similar to that followed by the permanent environmental variance (figure 1). Higher *rep* estimates are seen at the beginning and the end of the lactation, exactly as shown for permanent environmental variance estimates (figure 1). Gebreyohannes *et al* (2016) reported higher *rep* estimates that ranged from 0.84 at the beginning of the lactation to 0.94 at the last DIM, their trajectory pattern was equal to that of their h^2 estimates and this was consistently increasing as lactation progressed. Other estimates of *rep* for daily milk yield were not found in the literature reviewed in this study.

Genetic correlations presented in table 2 between the sixth and some selected DIM were all positive and high between adjacent test-days and, as expected, they tended to decline as the distance between them increased. However, the genetic correlation among the sixth and above 265 DIM began to increase (data are partially shown in the seventh column of table 2). The lowest genetic correlation was 0.37, estimated between the sixth and DIMs from 260 to 266. Gebreyohannes *et al* (2016) reported a constant and declining genetic correlation trend across all over the lactation but at a lesser extent, for instance, their lowest estimated genetic correlation was 0.78 between the 30th and the 300th DIM. Bignardi *et al* (2011) also described that genetic correlations were high for adjacent test-days and decreased with increasing distance between them, however, these authors obtained negative genetic correlations between the initial and final test-days with the four models

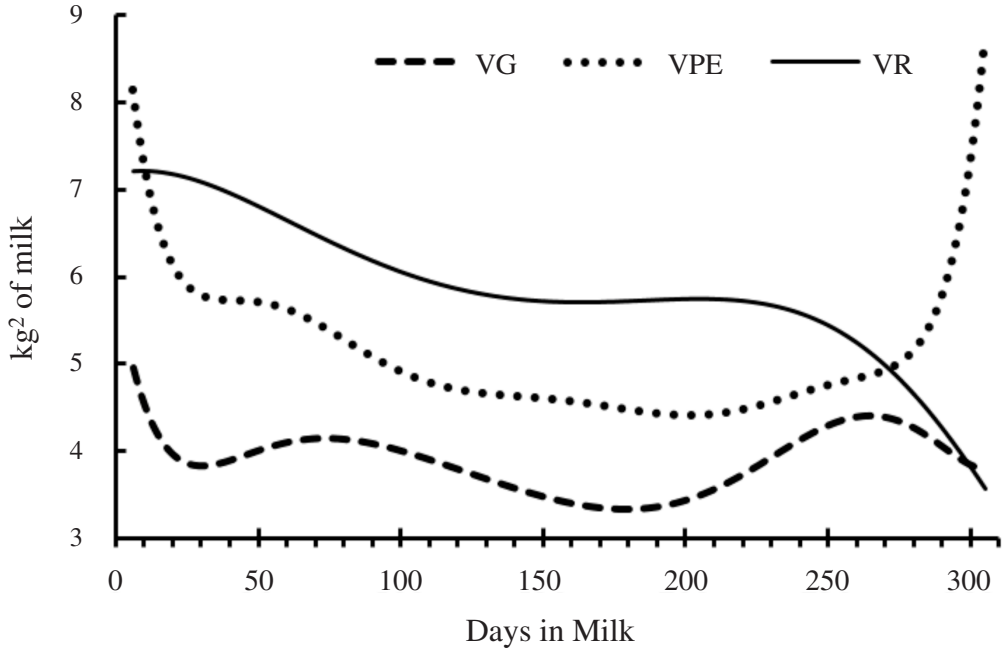

Figure 1. Changes of the genetic (VG), permanent environmental (VPE) and residual (VR) variance estimates from 6 to 305 days in milk.

used in their research, irrespective of the function used to fit the lactation curve. Bignardi *et al* (2011) attributed those negative correlations to the difficulty of modelling initial test-day milk yields because in this period the cow suffers from post-calving stress and a negative energy balance. Jamrozik and Schaeffer (1997) and Cobuci *et al* (2005) also reported negative genetic correlations among test-day milk yields, however, in both studies the random regression coefficients were not included to model permanent environmental effects.

The objective of this study was to estimate genetic parameters for daily milk yield using a random regression test-day model and data from the Los Ríos region in Chile. Although the methodology was accessible in Chile since 2001 (Uribe 2001), its implementation using Chilean dairy records has not been reported. During the last two decades, available computing technology[1] and model fitting strategies have improved dramatically and some studies in which different functions and models were compared are available in the literature reviewed here (Cobucci *et al* 2005, Bignardi *et al* 2011, Gebreyohannes *et al* 2016). To model the lactation curve, orthogonal polynomials were chosen in this study because their properties and advantages, compared to other mathematical functions, have extensively been documented (Schaeffer 2004, Bohmanova *et al* 2008, Bignardi *et al* 2011). In this type of model, each animal gets five regression coefficient estimates corresponding to its random additive genetic, expression of breeding values for a given animal in its i^{th} DIM as presented by Uribe (2001).

Test-day models using random regression have been implemented for routine genetic evaluation of dairy cattle in several countries, however, the Chilean dairy cattle industry has not implemented a public genetic evaluation system yet. The use of orthogonal polynomials in a random regression model to estimate heritability and repeatability in dairy cattle of Los Ríos Region, Chile, showed good results. Although the estimated genetic parameters did not radically depart from those reported in the Chilean literature, they are mathematically more precise, for estimating the true heritability and repeatability, than those calculated using adjustment factors suggesting that the model used in this study could be the starting point to develop a robust national genetic evaluation system for dairy cattle.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Ignacy Misztal, University of Georgia, USA, for allowing the use of the AIREML computer program. They also thank to Dr. Shogo Tsuruta, University of Georgia, USA, for providing valuable insights on fitting in the model heterogeneous residual variance.

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Austral J Vet Sci **52, 109-112 (2020)**

CASE REPORT

Clinical presentation and treatment of multifocal epitrichial sweat gland carcinoma in a horse

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ABSTRACT. Epitrichial gland carcinoma is a very rare type of skin tumour in horses. This report describes a horse presenting multiple nodules with associated normal, alopecic and ulcerated skin diagnosed via histopathology as epitrichial gland carcinoma. Treatment consisted of combined surgical excision, topical therapy for ulcerated nodules and cryotherapy for non-ulcerated tumours. Six months following therapy, the excised masses had not regrown and only 10 out of 25 small tumours previously treated with cryotherapy were noticeable.

Key words: horse, skin, apocrine glands, neoplasia.

The skin is the most common site of neoplasia in the horse, representing about 50% of all equine neoplasms (Scott and Miller 2011[a]). Among the tumours affecting equine skin, sarcoid, melanoma, papilloma and squamous cell carcinoma have been the most frequently identified (Scott and Miller 2011[a], Hewes and Sullins 2009). Less common skin tumours such as basal cell (Slovis *et al* 2001), glomus (Burns *et al* 2011), myxomas, and mast cell tumours (Lykkjen *et al* 2006) have been also diagnosed. Epitrichial or apocrine sweat gland carcinoma is a very rare condition that has been reported very few times and represents 0.1% of skin neoplasms of the horse (Anderson *et al* 1990, Scott and Miller 2011[a], Cihocki *et al* 2007, Ghasami *et al* 2017). Sweat gland neoplasms are tumours arising from the glandular or ductal components of epitrichial sweat glands being commonly benign proliferations.

A 12-year-old crossbreed mare (Holsteiner x Selle Français) developed a large number of cutaneous masses extending from just distal to the carpus to the pastern of the right forelimb. The masses developed over six-months and, concurrently, the mare developed lameness of the right forelimb that did not improve with anti-inflammatory treatment (phenylbutazone[1] 2.2mg/kg IV BID for 5 days). On presentation, the mare was bright, alert and responsive. Twenty-five skin nodules distal to the carpus with associated normal, alopecic and ulcerated skin surfaces were present (figure 1A). The nodules varied in shape and size ranging from 0.5 cm to 2.5 cm diameter. The differential diagnoses included equine sarcoid, eosinophilic granuloma, and chronic proliferative pastern dermatitis (“grapes”).

Three small masses were excised under standing sedation and were submitted for histopathological evaluation. None appeared to extend into the subcutaneous tissue grossly, and all of them were easily delimitated and removed. Submitted masses had similar histological features. Neoplastic cells were infiltrated in the dermis with formation of dense clusters with a glandular appearance (figure 2A, 2B). Trabecular, tubular and solid structures were observed with frequent central necrosis. Occasionally, luminal structures containing eosinophilic material were observed. Clusters were separated by fibrous connective tissue (desmoplastic stroma) (figure 2C). Neoplastic cells were cuboidal with eosinophilic cytoplasm with clearly defined borders. The cells presented round to oval, central and hyperchromatic nuclei with inconstant nucleoli (figure 2D), and 1-2 mitosis/x400 microscopic field were observed. The final histopathological diagnosis was epitrichial sweat gland carcinoma.

Following excision, the surgical sites were closed using 2-0 polyglicolic acid suture, and the limb was bandaged. The ulcerated nodules were lavaged with Chlorhexidine 2%[2] daily for two weeks, and silver spray[3] was applied daily for two weeks. Antibiotic therapy with Procaine Penicillin G + Dihydrostreptomycin[4] (12.000 UI/kg IM SID) for 10 days and Gentamicin[5] (6.6 mg/kg IV SID) for 10 days was given to treat the presumed infection of the ulcerated skin of the palmar aspect of the pastern, and Flunixin Meglumine[6] (1.0 mg/kg IV BID) was administered for 5 days to minimise pain and inflammation. Cryotherapy[7] was used to treat the remaining non-ulcerated tumours. Cryotherapy was applied via a commercial system which uses dimethyl ether, propane, and isobutane to treat papillomas in humans. This system is an easy and ready-to-use

Received: 21.01.2020.

Accepted: 01.07.2020.

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1 Equs 10%, Drag Pharma, Santiago, Chile

2 Clorhexidina Gluconato 2%, Difem Pharma, Santiago, Chile

3 Curabichera Plata, Mustad, Buenos Aires, Argentina

4 Pencidrag, Drag Pharma, Santiago, Chile

5 Gentamicina 10%, Veterquimica, Santiago, Chile

6 Ehliglumina 8,4%, Centrovet, Santiago, Chile

7 Pointts®, Genomma lab, Santiago, Chile

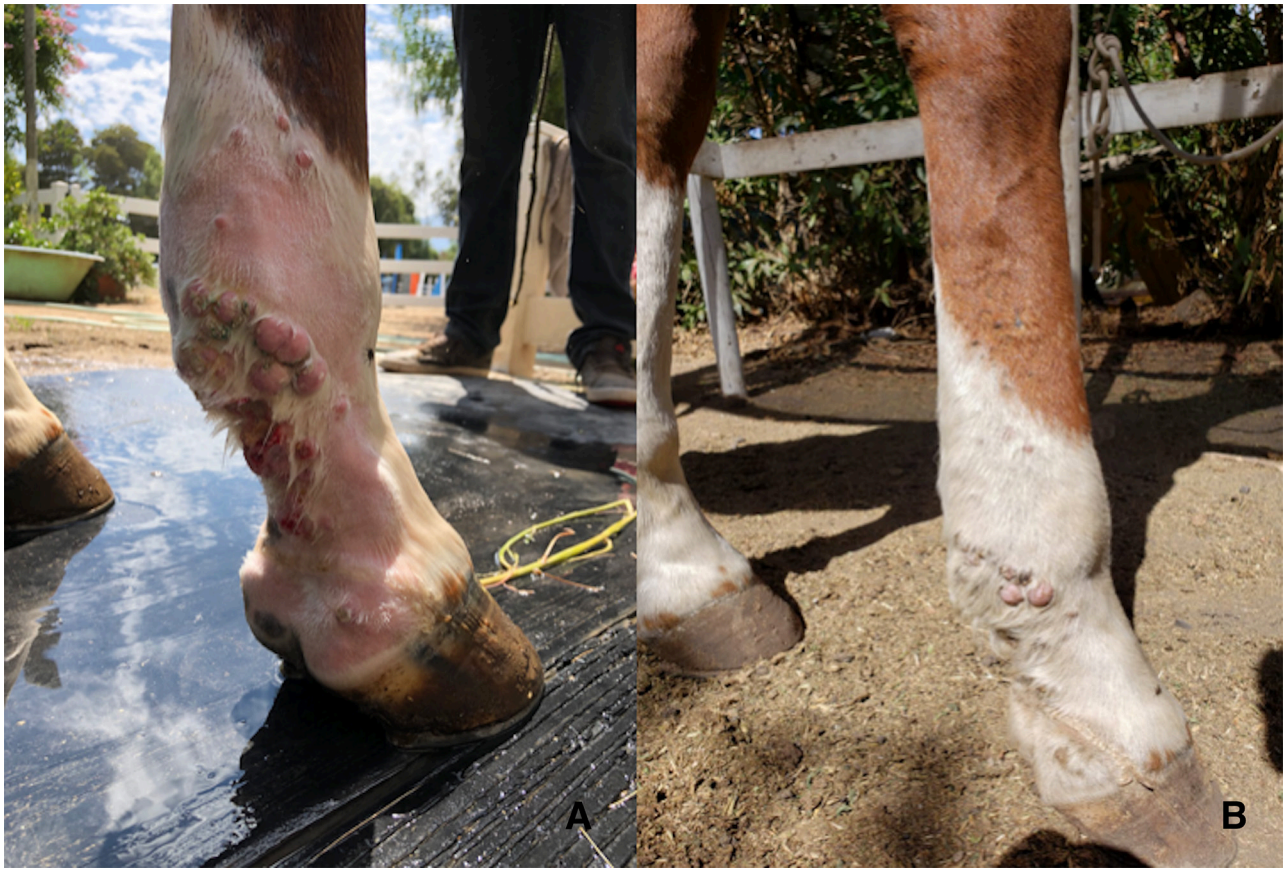

Figure 1. Right forelimb palmar fetlock and pastern of a 12-year-old mare. **A)** Multiple alopecic and ulcerated skin nodules; **B**) 1-month follow up after cryotherapy. Despite the presence of hair regrowth somewhat obscuring the complete visualisation of the masses in the photo, the larger nodules are significantly smaller, and many of the smaller tumours are undetectable.

product which consists of spray and sponge applicators that are applied directly over the affected skin according to manufacturer's instructions.

Re-examination one month after initialising the treatment showed that the ulcerated pastern skin had completely healed, and no lameness was evident. The surgery sites displayed no abnormalities, and normal scar tissue was present. The remaining 10 masses detectable after cryotherapy were smaller, and no new tumours were observed (figure 1B). Since improvement was noted and no adverse reactions nor abnormalities were detected during the initial treatment, a second course of cryotherapy was recommended but it was declined by the owner. Six months following therapy, the excised masses had not regrown, and only 10 out of 25 small tumours previously treated with cryotherapy were noticeable.

We described a case of epitrichial sweat gland carcinoma with a good outcome following treatment with excision, topical therapy, cryotherapy, and conservative medical therapy. The epitrichial sweat glands are distributed throughout all haired skin, and they are largest and most numerous in the submandibular region, mane, near the mucocutaneous junctions, and near the coronary band (Scott and Miller 2011[b]). It is therefore unsurprising that the tumours described previously have been located in the genital tissue and pastern region. In our report, the fetlock region tumours were larger and more densely arranged, whereas the main characteristic of the pastern region tumours was ulceration. Finally, the tumours located in the metacarpal region were smaller and had normal or alopecic skin.

The presented case, although similar to those reported by Cihocki *et al* (2007) and Ghasemi *et al* (2017), is unique because of the variable nature of the lesions, the treatment selected and the tumour behaviour after treatment. Furthermore, since the location, type, and number of lesions in our case differs from previously described cases, this report adds to the knowledge of this rare and so far poorly understood type of tumour in horses.

The diagnosis was achieved by histopathology and was based on the appearance and description of epitrichial sweat gland carcinoma published in previous reports (Kalaher *et al* 1990, Cihocki *et al* 2007, Ghasemi *et al* 2017). All samples had the same architecture suggesting that one tumour may spread throughout the limb. Although haematogenous or lymphatic spread has been suggested

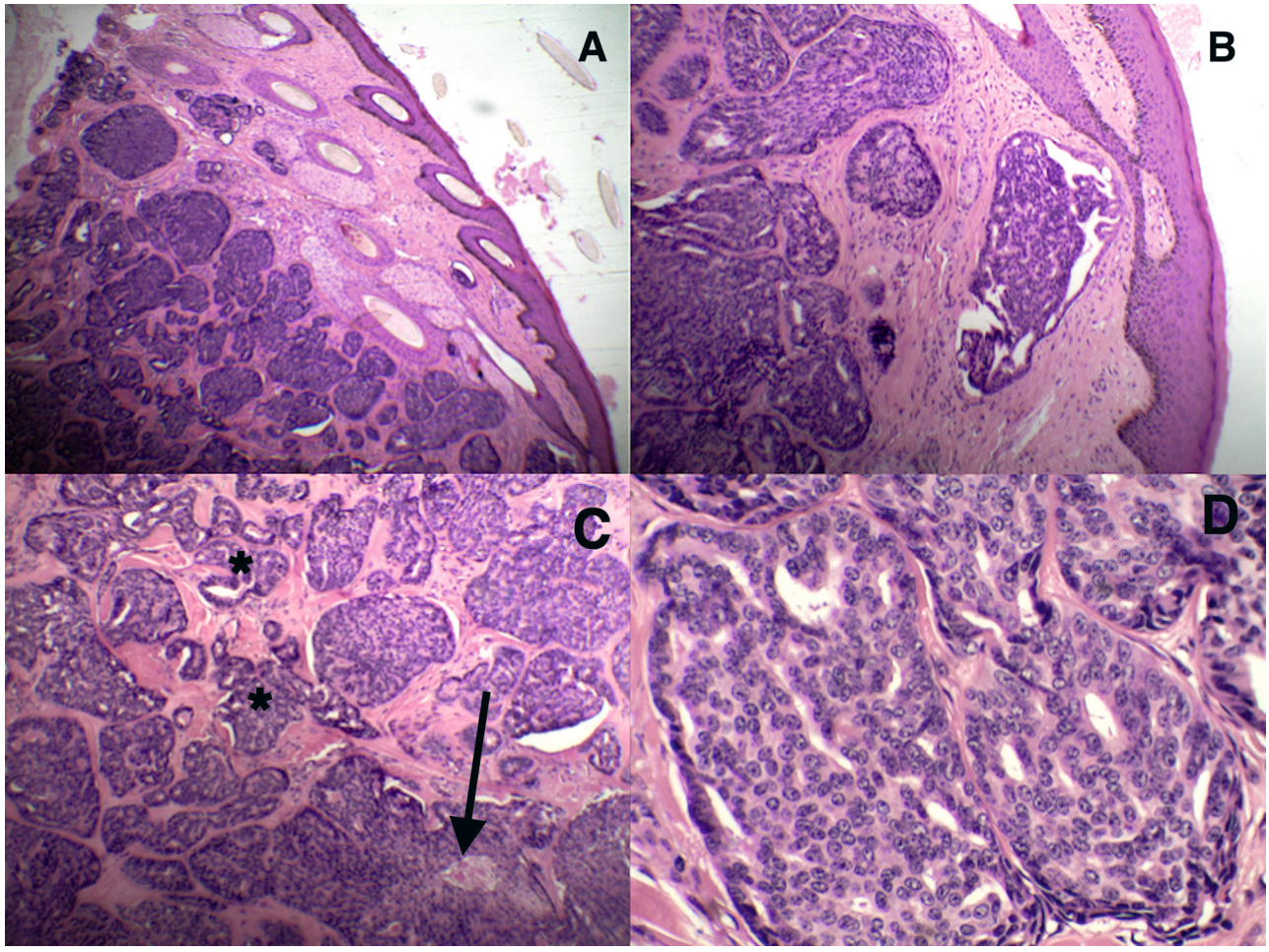

Figure 2. Histological sections of a skin nodule obtained from the fetlock area of the right forelimb, haematoxylin and eosin stain. **A**) Neoplastic cells infiltrated into the deep dermis (10x); **B**) Densely packed clusters of neoplastic epitrichial sweat gland cells demonstrate irregular, infiltrative growth (40x); **C**) Poorly demarcated and infiltrative clusters of neoplastic cells form numerous tubular (ducts) (*), trabecular and solid structures, the clusters are separated by fibrous connective tissue (desmoplastic stroma). A focal area of necrosis is present within one cluster (arrow) (100x); **D**) Note the presence of cuboidal cells with eosinophilic cytoplasm in the neoplastic glandular epithelium, the cells contain round to oval central and hyperchromatic nuclei with inconstant nucleoli (400x).

in humans, dogs and cats (Kalaher *et al* 1990, Simko *et al* 2003, Tlemcani *et al* 2010, Jark *et al* 2015), to date there is no evidence supporting that notion in horses. The mechanism of spread is still open for discussion given that some authors report that apocrine neoplasias have invasive behaviour, and they can metastasize to the capsule and stroma, blood vessels, and distant organs in humans (Tlemcani *et al* 2010) and dogs (Simko *et al* 2003), while other authors suggest that apocrine adenocarcinomas invade lymphatics without any distant metastasis (Kalaher *et al* 1990, Haziroglu *et al* 2012, Jark *et al* 2015). Despite the information available in other species, neither blood nor lymphatic vessel invasion was observed in histological specimens from our horse, which is similar to what was observed in previous reports (Cihocki *et al* 2007, Ghasami *et al* 2017). Although spread via the lymphatic route cannot be completely ruled out in our case, the lack of supporting evidence of spread via blood or lymphatics in horses and the lack of vessel invasion in the histological samples of this case support the possibility of *de novo* development of multiple tumours.

Treating equine skin neoplasia has been historically challenging, and unresponsiveness to treatment, tumour re-growth and metastasis are frequent situations affecting the effectiveness of the treatment and the outcomes (Hewes and Sullins 2009). Cisplatin administration, either mixed with sterile sesame oil or slow-release biodegradable beads, has shown good outcomes after treatment in different types of equine skin tumours (Hewes and Sullins 2006), however, treatment options for epitrichial sweat gland carcinomas have been limited mostly to surgical excision and cryosurgery (Scott and Miller 2011[a], Ghasami *et al* 2017). Recently, Cihocki *et al* (2007), after a failed treatment with cisplatin, used an FDA approved topical

cream (Imiquimod) to treat a horse presenting sweat gland carcinomas. However, Imiquimod cream showed some side effects throughout the treatment course such as marked inflammatory response at the application site and only 50% efficacy (Cihocki *et al* 2007). Adverse side effects associated with topical imiquimod were also described in a sarcoid pilot study, however, the efficacy of the drug appeared to be higher for this type of neoplasia (Nogueira *et al* 2006). The horse in this report was treated with a cryotherapy system that contains dimethyl ether, propane and isobutane (Pointts®), and is commonly used for the treatment of verrucous lesions in humans. The use of this product appeared safe and was not associated with significant side effects. The remaining nodules (10) were significantly smaller after one treatment with cryotherapy. A second and third treatment with cryotherapy as labelled for humans was indicated to achieve full remission of tumours, but the owner declined to continue with therapy due to the good response to initial treatment, musculoskeletal soundness, and return to full work. Considering the efficacy of the cryotherapy to resolve and/or shrink this type of tumour and the anti-tumour properties previously described for Imiquimod 5%, a combined therapy could be considered to achieve full remission of this type of carcinoma.

In conclusion, epitrichial sweat gland carcinomas should be considered as a differential diagnosis when tumours with normal, alopecic and/or ulcerated skin are present in the pastern, fetlock, and even near the carpal region. This type of tumour does not normally cause lameness, nonetheless, when ulcerated lesions are present, they can get infected and cause musculoskeletal discomfort. The few cases described so far have demonstrated this type of neoplasia to be locally invasive but without resulting in metastasis as stated in other species. Treating skin tumours has been historically challenging, however for this specific type of tumour, surgical excision and cryotherapy with dimethyl ether, propane and isobutane preparation appear to be effective without side effects attributable to the treatment. Finally, further studies are needed to elucidate the pathways of spread throughout the body for this type of tumour, the mechanism of which is yet unknown in horses.

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Austral Journal of Veterinary Sciences publishes in English, original scientific contributions such as scientific articles, reviews, short communications and case reports which can include clinical observations, descriptions of methods or techniques and advances in all aspects of veterinary science and animal welfare.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be submitted to the Editor via the on-line platform www.australjvs.cl and must include:

- An electronic version of the text (MS Word format), tables (preferably in MS Word format), charts (Excel format), figures and photographs (TIFF, 300 dpi)
- A cover letter to the Editor signed by the corresponding author should include the following:

A declarative statement that the manuscript represents new information that has not been previously submitted or published elsewhere; or an explanation of any previous publication or presentation of all or parts of the manuscript.

A declarative statement that all authors of the paper have read and approved the final version of the manuscript submitted and that all have made substantive contribuionts to the work. The statement must include the email address of all the authors.

Specification of the type of manuscript that is being submitted.

A description of how the information provided in the manuscript is original, new, timely, significant, and relevant to the readers of *Austral J Vet Sci*.

- The manuscripts must be original, unpublished and may not be considered for publication in another journal.
- Manuscripts that do not conform to editorial requirements will be returned without review.
- For manuscripts describing studies involving animals or humans, the appropriate Bioethical Committee Certification must be mentioned under Material and Methods.
- Referees of Austral Journal of Veterinary Sciences will aid the Editorial Committee to determine whether the manuscript fulfills publication requirements. The authors must suggest at least three referees. All articles submitted for publication will be assessed by two referees. The referees will be selected by the Editorial Committee, and may or may not include those nominated by the authors. In the case of a disagreement between the referee's reports, a third referee will aid the Editorial Committee to reach a decision. Referees are obliged to keep all information from the articles confidential, including unpublished information. Authors should state any potential conflicts of interest at the time of submission of the manuscript. Such information will not alter established editorial and review policies but will assist the editorial staff in avoiding any potential conflicts that could give the appearance of a biased review.
- The final decision regarding acceptance of the manuscript will be taken when the Editorial Committee accepts the manuscript following correction according to the referees' comments.
- Accepted articles must pay a publication fee prior to publication, the amount of which can be found at www.australjvs.cl/ajvs/web-pay/.

PREPARATION AND FORM OF MANUSCRIPTS

Type of articles

Review articles: provide expert summaries of current knowledge in a particular field of veterinary science, and do not necessarily have a set format. Authors should consult with the Editor before initiating a review. The Editorial Committee may solicit an expert to prepare a review, which will also be refereed and edited. Reviews must not exceed 30 pages in length, including tables, figures and references.

Scientific articles: report new advances in veterinary science based on original research. The format must include abstract, introduction, material and methods, results, discussion, acknowledgements (when pertinent) and references. The maximum length of the manuscript is 20 pages, including tables, figures and references.

Short communications: briefly inform of an advance, experimental result, new methodology, with the following format: abstract, introduction, material and methods, results and discussion (combined), acknowledgements (when pertinent) and references. The maximum length of the manuscript is 12 pages, including tables, figures and references.

Case report: is a brief note that describes preliminary findings and contributes significantly to the understanding of the Veterinary Science. The maximum length is 1,300 words which includes the main body of the text and cites. An abstract of 50 words is required, plus 15 references and two tables or figures, or one of each. Acknowledgements can be included (when pertinent). Subtitles must not be used to divide the main body of the text.

JOURNAL STYLE AND LAYOUT

General presentation: Manuscripts must be written using 12 point Times New Roman font with one and a half-line spacing, on one side only of letter paper (21.5 x 27.9 cm) using 2 cm margins on all sides. Pages must be numbered consecutively in the top right corner, and lines must be numbered on the left,

starting with number one, on all pages. The main body of the text must be indented.

Headings must be in upper case, left-justified on a separate line with no full stop following, e.g. MATERIAL AND METHODS. Only the first letter of sub-headings is capitalised. Primary sub-headings (e.g. Experimental design) should be left-justified; secondary sub-headings are left-justified and italicised. Do not use underlining and do not number sub-headings or itemised lists.

In the text, numbers must be written in numerals. When a sentence begins with a number or when necessary for clarity, this should be written in words. A decimal point must be preceded by a number (e.g. 0.5 not .5). All measurements must be reported in SI units (www.nist.gov/pml/pubs/sp811/) unless it is normal practice in a discipline to use derivatives (e.g. the Curie international unit). Dates must be formatted as 07 September, 1954 in the text, but they may be abbreviated in tables and figures. Use the 24-hour clock for times of day (e. g. 13:00 h). Chemical nomenclature must be expressed using the Biochemical Society Standards (Biochem J 209, 1-27, 1983), generic names (in lower caps) must be used for medications. If brands and sources of medications need to be included, this should be included as a foot-note. Enzymes must be identified at first mention, in accordance with the Enzyme Commission of the International Union of Biochemistry. Latin terminology and abbreviations commonly used in scientific literature, such as *in vitro*, *in vivo*, *ad libitum* must be italicised. Scientific names of animal species should be mentioned once in the text, complete and in brackets, subsequently only the common name should be used. Probability values must be presented as $P<0.05$ or $P<0.01$. Standard deviation, standard error of the mean and confidence intervals are abbreviated as follows: SD, SEM and CI, respectively.

Title

Title must be short, specific and informative. The title is centred in bold, starting at line 10 without using trade names or abbreviations. Only the first letter is capitalised. Scientific names of animal species must be mentioned in the tittle, in brackets, only in the case of non-domestic species.

Author's names and addresses

Author's names are written underneath the title, separated by a space. Use full name and separate authors by commas, as in the example: Christopher A. Westwood, Edward G. Bramley, Ian J. Lean. Superscript letters should be used after each author's name to identify the section, department, service or institute, city and country of the author where the work was conducted. The corresponding author is indicated using the superscript letter followed by an asterisk, with the telephone, mailing and email addresses indicated in the footnote.

Footnotes

These are used to indicate a web address (URL) and to define abbreviations used in table titles, commercial brands, the name and address of companies. They must be indicated with numbers.

Abstract

The second page must contain an abstract of no more than 250 words that describes the objectives of the study or research, the material and methods used, the principal results and the most important conclusions. Non-standard abbreviations must not be used. On a separate line, left-justified, and separated by a space, up to four Key words should be identified. The use of key words containing more than two words (a phrase) must be avoided.

Introduction

The subheading "Introduction" is written on the next page following the Abstract and Resumen. In the following line, indented by 5 spaces, the context of the study is briefly presented without an extensive revision of the theme, and only citing the most relevant references. The hypothesis and objectives of the study must be clearly and concisely presented.

Material and methods

Separated by one space from the previous section, this section should contain sufficient detail to allow others to repeat the study. When the first reference in the text is made to medications or chemicals, the generic name, dose and route of administration should be indicated. For specialised equipment, the brand, model and manufacturer's name must be indicated. Studies involving animals or humans must mention the appropriate Bioethical Committee Certification. Details of all statistical methods used must be given at the end of this section under the sub-heading "Statistical analysis" and should include adequate detail to allow readers to determine precisely how data have been analysed and the units that are used to express the results (mathematical mean, standard deviation, standard error of the mean, mediums, ranges or confidence limits, etc.). The use of parametric (Chi-square, student's t-test, ANOVA, etc.) or non-parametric (Wilcoxon, Kruskal-Wallis etc.) analyses must be indicated. The name, version and sources of computational statistical analysis programs must be identified, e.g. SPSS 9.0 (SPSS Inc, Chicago IL, USA).

Results

Separated by one space from the previous section, this section should contain a concise and logical description of the results obtained without discussion or reference to other work. The results can be supported by tables and/or figures that present the pertinent data without repetition, and data presented in tables and figures should not be repeated in the text. In the case of Original articles only, this section and the Discussion are separated.

Discussion

This section should evaluate and interpret the results and relate these to other relevant results. The results should not be repeated and new results must not be presented in this section. Care should be taken to ensure that the discussion is developed in a logical and concise manner, and conclusions are reached, as well as a discussion of their relevance. Conclusions that are not directly supported by the data of the study or other unpublished studies should not be presented.

Acknowledgements

This section should be brief, and should only include people or institutions that have made a direct contribution, provided necessary

material or have provided the facilities for the study's development. The source of funding should be indicated in this section.

References

The accuracy of the reference section is the responsibility of the authors and references must be verified against the original article. Please ensure that all articles cited in the text are included in the reference list and vice versa. In the main text, citations should be listed in parentheses in chronological order, citing authors' names, and using *et al* after the first author's name where there are more than two (e.g. Smith 1994, Castro and Martínez 1996, Weiss *et al* 2002).

All lines after the first line of each entry in the reference list should be indented 0.5 cm from the left margin (hanging indentation). The reference list must be ordered alphabetically according to the first author's name, and all authors' names and initials must be included. When no author is given, use the term "Anonymous" in both text and reference list. References with the same author, single or with coauthors, should be listed in chronological order. If there were more than five authors, *et al* must be used after the fifth one. The letters a, b, c, etc. should be appended as a superscript when more than one work is cited from the same author within the same year. Author names should appear with the initials and first letter of the surname in upper caps and the remainder of the surname in lower caps, with no periods between initials. Journal title abbreviations and names of books must be in italics. For journals, ISI abbreviations must be used. The following examples can be used as a guide:

For journal articles:

Mella C, Medina G, Flores-Martin S, Toledo Z, Simaluiza RJ, *et al*. 2016. Interaction between zoonotic bacteria and free living amoebas. A new angle of an epidemiological polyhedron of public health importance?. *Arch Med Vet* 48, 1-10.

Neverauskas CE, Nasir A, Reichel MP. 2015. Prevalence and distribution of *Neospora caninum* in water buffalo (*Bubalus bubalis*) and cattle in the Northern Territory of Australia. *Parasitol Int* 64, 392-396.

For books, chapters in books or occasional publications:

Leeson S, Summers JD. 2005. *Commercial poultry nutrition*. 3rd ed. Nottingham University Press, Nottingham, UK.

Larson V. 2009. Complications of chemotherapeutics agents. In: Silverstein D, Hopper K (eds). *Small Animal Critical Care Medicine*. Saunders Elsevier, St Louis, Mo, USA, Pp 817-820.

WHO, World Health Organization. 1972. International Drug Monitoring: The role of national centres. *Tech Rep Ser* WHO Nº 48.

SAG, Servicio Agrícola y Ganadero, Chile. 1996. Resolución Exenta Nº 3599 del 29 de noviembre de 2006.

For softwares:

SAS, Statistical Analysis System. 2000. *SAS version 6.0*. SAS Institute Inc., Cary, NC, USA.

R Core Team. 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

For articles and proceedings published in regular series:

Zimbelman RB, Rhoads RP, Rhoads ML, Duff GC, Baumgard LH, Collier RJ. 2009. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. *Proceedings of the 24th Southwest Nutrition and Management Conference*, Tempe, Arizona, USA, Pp 158-169.

For PhD and MSc dissertations:

Lindberg A. 2002. Epidemiology and eradication of bovine virus diarrhea virus infections. *PhD Dissertation,* Swedish University of Agricultural Sciences, Uppsala, Sweden.

Minimise the citation of abstracts as references. Authors are specifically discouraged from citing "unpublished data" or "personal communication", unless this information exists in written form, in which case the text should be referred to as a footnote, but this should not appear in the list of references. References to papers which have been accepted but not published should be cited as "in press", whereas manuscripts which have been submitted for publication but not accepted should be referred to as "unpublished data".

Web pages should not be included as references. If required, web page addresses should be written as footnotes, including date of consultation.

COMPLEMENTARY INSTRUCTIONS

Tables

The titles to tables and figures should be self-explanatory. The number of tables should be kept to a minimum and presented on separate pages with their respective titles at the top. Information in tables must not be repeated in the text. Tables must be numbered consecutively with Arabic numbers in the order in which they are referred to in the text. The brief title to the table should indicate the contents of the table and should be understandable without reference to the text. Each column of each table must have a short or abbreviated heading. Only column headings and general titles should be separated with horizontal lines. Data columns should be separated by spaces and not vertical lines. When additional explanatory information is required, this should appear at the foot of the table. Explanatory information for non-standard abbreviations and units should appear within parentheses. If superscripts are used to indicate significant differences between values, use a, b, c. Minimise the number of digits in each column. Indicate a zero value as 0. Table widths should not exceed 80 mm for one column or 170 mm for two columns.

Figures

Figures should be submitted on separate pages, with their respective titles in English at the bottom and numbered consecutively using Arabic numerals in the order they are referred to in the text, e.g. Figure 1, not Fig. 1. Figures include all illustrations that are not Tables, e.g. graphs, radiographies, ecographies, electrocardiograms, photographs, etc. Figures must be vertically oriented and be accompanied by a short descriptive caption that contains an explanation for all markers, lines and symbols used but no abbreviations. If the figure contains sections, these should be labelled as a, b, c, etc. in the top right corner and must be described in the caption. Figures may be one or two column-widths (80 or 170 mm, respectively). The authorship of non-original figures must be acknowledged, and

when appropriate, authorisation to reproduce these figures must be provided.

Changes to authorship

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended.

Proofs

A proof will be sent to the corresponding author for proofreading in PDF format, and must be returned within the specified time, otherwise the Editor reserves the right to carefully proof-read the article but without assuming responsibility for errors, to continue with the publication process. Alterations to the proof that do not correspond to minor errors will be charged to the authors. Neither the Editor nor the Publisher accept any responsibility for printed errors that had not been indicated by the authors.

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VOLUME 52 / VALDIVIA - CHILE / 2020 / Nº3