Genetic study on wet carcass syndrome

10/08/2018

Detection of quantitative trait loci affecting wet carcass syndrome in sheep

Industry Sector: Cattle And Small Stock

Research Focus Area: Animal Products, Quality And Value-Adding

Research Institute: Agricultural Research Council – Animal Production Institute

Researcher: Lené Van Der Westhuizen

The Research Team

Title	Initials	Surname	Highest Qualification
Prof	M.D.	MacNeil	Ph.D.
Prof	M.M.	Scholtz	D.Sc.
Prof	M.D.	MacNeil	Ph.D.
Prof	F.W.C.	Neser	Ph.D.
Prof	A.	Maiwashe	Ph.D.
Mrs	A.	Theunissen	M.Sc.
Ms	M.	le Roux	M.Sc.

Year Of Completion : 2018

Aims Of The Project

- To map quantitative trait loci affecting wet carcass syndrome.
- To identify specific loci affecting the predisposition to wet carcass syndrome (detection of a major gene).
- To develop a diagnostic test for the genetic predisposition to wet carcass syndrome (if a candidate gene can be identified as the cause).
- If a major gene is not responsible for wet carcass syndrome the second phase of the project will have the aim to develop a polygenic prediction equation for the predisposition of sheep to wet carcass syndrome.

Executive Summary

Wet carcass syndrome (WCS) is a condition predominantly found in sheep, which negatively affects the quality of their carcasses. During the pre-slaughter period, the animal appears to be clinically normal, showing no symptoms of an abnormality. However, after the removal of the skin during the slaughter process the carcass appears to be “wet”. When the description and results of prior research are taken into account, no physiological, environmental or management system was conclusively identified as the causative agent of WCS. Previous research has also not considered a potential genetic basis for WCS or the potential for an interaction of genotype with the environment (stress). Furthermore, the tentative breed-specificity, i.e. Dorper sheep breed, of the condition lends some credence to a potential genetic basis for it. The current study employed the Ovine Infinium® HD SNP BeadChip and a genome-wide association analysis approach to scan the genomes of both afflicted- and unafflicted sheep in search of putative quantitative trait loci associated with the WCS phenotype. This study was not only one of the first in Southern Africa to make use of this specific BeadChip but also the first to investigate the role of genetics as a causative factor of WCS. Muscle samples from sheep carcasses (33 afflicted and 36 unafflicted) were collected from three different abattoirs.

Using a candidate gene approach it was possible to map genetic loci, *RYR1* (Chromosome 14) and *PRKAG3* (*RN*$^{-}$; Chromosome two) causative of phenotypically similar conditions such as porcine stress syndrome and red, soft and exudative meat to the ovine genome, respectively. The positions of these loci mapped to the ovine genome were not in accordance with the loci showing significant association with the WCS phenotype; and no relationship was found between single nucleotide polymorphisms located within these genes and WCS. Furthermore, along with the latter approach, the test of runs of homozygosity presented similar results as well as providing plausible evidence that WCS is not a recessive inherited condition.

To test for an association between the phenotype (WCS) and a genetic marker(s) i.e. SNPs, a case-control study design was implemented. Given the relatively small sample size of the current study, the results obtained from the GWAS attested strong evidence of at least two loci, oar3_OARX_29903534 and oar3_OARX_113973214 positioned within the non-homologous region of the X chromosome for WCS carcasses. All afflicted animals, both males and females, carried at least one allele for marker oar3_OARX_113973214, which was shown to be related to the WCS phenotype. On the contrary, some of the unafflicted animals also carried this specific allele. Given the apparent influence of stress on WCS, these unafflicted males and females in all likelihood did not experience adequate levels of stress to manifest the condition post-slaughter. The results of the current study also indicated that WCS may possibly be a rare X-linked inherited condition, provided only female individuals are considered. Finally, two possible major loci involving two major genes, *HTR2C* and *DMD*, positioned on the non-homologous region of the X chromosome have been identified as novel positional and functional candidate genes for WCS in sheep.

Popular Article

Wet carcass syndrome (WCS) is a condition mainly found in sheep, which negatively affects the quality of their carcasses. It has been identified in both sheep and cattle breeds, however, the frequency of WCS seen in cattle is substantially less than in sheep. Despite long-standing knowledge of the condition and research, little more is known about wet carcass syndrome and its causes than when it was discovered some three decades ago. Furthermore, it is very difficult to simulate the condition and in some years it is almost absent. Possible causing factors of WCS included the over-hydration of thirsty sheep on arrival at abattoirs, transport distances to abattoirs, allergies, compulsory dip, washing of carcasses in abattoirs under high pressure, condensation in coolers and provision of feed blocks during the pre-slaughter phase.

However, research could not find any link between these factors and the occurrence of WCS. Therefore, when the description and results of prior research are taken into account, no physiological-, environmental- or management system was conclusively identified as the causative agent of WCS. However, stress experienced by the animals during the pre-slaughter period has been identified as a possible contributing factor. Some prevention strategies have been proposed, but the problem still appears from time to time and is more severe in some years.

Wet carcass syndrome is mainly observed in hairy-type Dorper sheep and crosses of Dorper with indigenous and locally developed breeds of South Africa and Namibia, and largely seen in A0 / A1 carcasses (very low fat content with poor conformation). The Dorper breed is greatest in numbers in the studied areas (geographic regions where WCS occurs most frequently) of the Northern Cape Province in South Africa and the southern part of Namibia (Kalahari dunes and sandy veld). Unofficial slaughter statistics from WCS afflicted areas, reveal that certain abattoirs have higher numbers of WCS carcasses, whereas other abattoirs in the same region will have no recorded incidences. Communication between the researcher and abattoir management exposed the seriousness of the condition to communities in the

Northern Cape. The condition is found widespread across areas where the grazing quality is poor, although the quantity is often abundant. WCS is also more frequently observed during autumn and winter, especially after droughts or after periods of above-average rainfall during spring, followed by low rainfall during the rest of summer.

During the pre-slaughter period, the animal appears to be physically normal, showing no symptoms of an abnormality. However, after the removal of the skin during the slaughter process, the carcass appears to be “wet”. An uncoloured, slightly sticky, jellylike fluid gives the carcass the shiny and wet appearance. The areas most affected on the carcass are the brisket, flanks, hindquarters, sides, and back. Affected carcasses do not dry off with overnight cooling. Consequently, WCS carcasses are not accepted, with two of the main reasons being appearance and a reduced shelf life. The most sensible explanation for the reduced shelf life is that the surface of the meat is a favourable environment for the growth of microorganisms. In addition, there is an occupational hazard associated with cutting wet carcasses in that a band saw pulls more on the meat which may result in injury to the operator. These observations further illustrate how potentially detrimental WCS is to the sheep meat industry in South Africa.

Lamb producers are very concerned about this condition and are actively participating in research to find solutions for this condition and to identify management procedures to alleviate their economic losses which may collectively rise to 10’s of millions of Rand annually. Carcasses that show WCS characteristics are generally rejected at the abattoir and not sold for human consumption. Taking carcass prices and inflation into account, the loss due to WCS can be estimated at a minimum of R 45,696,774 and during 2010 alone at R 27,010,387.

The literature review showed both promising results in terms of research opportunities and the identification of possible candidate genes for WCS. These candidate genes are the ‘genetic foundation’ of animals that will produce meat with characteristics of being pale, soft and exudative- (PSE); red, soft and exudative- (RSE) and dark, firm and dry (DFD) meat. These conditions are primarily observed in pork meat, but show phenotypic (visual) characteristics that are similar to WCS. All three of these meat characteristics are ‘trigged’ by stress. PSE/RSE meat will be the result of short term stress. Short term stress will cause a rapid decline of glycogen reserves within the muscle and finally result in meat with a low pH. The opposite occurs with DFD which is caused by long term stress. Long term stress causes severe muscle glycogen depletion, which in return causes the meat to have higher than normal pH levels. Selecting swine for leaner and heavier muscles resulted in some animals having greater susceptibility to stress and meat that is of poor quality. High vulnerability to stress in pigs is today referred to as porcine stress syndrome (PSS), and results in PSE meat. PSS can be described as acute death caused by stressors such as exercise, fighting, high temperatures, birth, stocking density, loading, transport, overcrowding at abattoirs, the use of electric prodders and abuse.

From a genetics perspective, PSS and RSE are caused by mutations within genes. PSS is caused by a single recessive inherited gene, *ryanodine receptor 1* (*RYR1*), located on Chromosome 6 of the pig genome. There have been reports on PSE meat in other species including cattle, ostriches, turkeys and chickens. The *Rendement Napole* (*RN*$^{-}$) gene is a dominant inherited gene and located on Chromosome 15 of the pig genome and will result in RSE meat. RSE meat will result in meat having a high drip loss.

The most recent research, finished in 2018, was the first study to examine the role of genetics as the leading cause of WCS. Three scientific methods were used to identify regions within the sheep genome that may contribute to the development of WCS. These methods are termed comparative genomics using candidate genes, runs of homozygosity (ROH) and a genome-wide association (GWAS) using a case-control study design. The first two methods did not provide the research team with any positive results. Firstly, the mutations within genes causing PSS and RSE in pigs most likely do not cause WCS. Secondly, an individual with identical long stretches of DNA that are inherited from parent lines is called runs of homozygosity. The research team searched for these ROH within the DNA of WCS affected carcass, but could not find any positive results.

The final phase of the study, i.e. GWAS, compared the DNA of both affected (WCS) and unaffected (normal) carcasses in search of DNA markers, named single nucleotide polymorphisms (SNPs), that might be associated with the WCS phenotype. When using the GWAS methods, an SNP will be associated with the condition when this specific genotype (genetic make-up of the animal) is more common in affected- than in unaffected carcasses. The results from this part of the study however, provided strong positive results that at least two of these DNA markers positioned on the X chromosome of the affected carcasses are most likely associated with WCS. However, these DNA markers were also found within the genotype of some of the unaffected or normal carcasses. Now to summarize the important results, some sheep carcasses that were normal also carried the same DNA markers than WCS affected carcasses. One possible explanation could be that these unaffected animals did not experience high enough levels of stress before slaughter to cause the WCS condition after slaughter.

These two DNA markers that were identified by the research team were then further linked to two genes, *5-hydroxytryptamine (serotonin) receptor 2C* (*HTR2C*) and *Duchenne muscular dystrophy* (*DMD*). As a result, these two genes were identified as candidate genes for WCS. Many biological functions of these genes exist, however, only a few functions could be connected to WCS. Assuming the *HTR2C* gene causes WCS, a disruption in cell homeostasis will occur, either during before the slaughter process by means of stress and anxiety; or after the slaughter period has been completed, through the calcium ion homeostasis mechanism within the cells of WCS affected muscles. Similarly, assuming the *DMD* gene causes WCS, the phenotype could be due to an increase in porousness of the cell membranes of muscles causing the typical shiny wet appearance of WCS. A novel or new porcine stress syndrome was, also identified in 2012 that is also caused by the *DMD* gene. Both of these genes explained in more modest words, will cause the cells within the muscle to act abnormally and fluid will move out from the cells onto the surface of WCS carcasses. However, this is only a theory and the precise biological mechanism causing WCS is presently unknown.

Future studies will first attempt to determine the exact position of the DNA marker(s) that cause WCS. Under the condition that WCS is caused by a single mutation, the development of a diagnostic test to identify live carrier animals of wet carcass syndrome, will enable sheep farmers to use this information in an attempt to eradicate the condition from their flocks. It is entirely possible that previous research attempts in search of environmental 'triggers' or causing factors for WCS were unsuccessful due to the unintentional sampling of mostly non-genetically susceptible or normal animals. Therefore, given the information provided and modern research techniques, nutritional studies will have the ability to make use of the genetically susceptible (WCS) animals to optimistically mimic WCS.

Please contact the Primary Researcher if you need a copy of the comprehensive report of this project – Lené van der Westhuizen on PienaarL@arc.agric.za

Animal Products, Cattle and Small Stock, Quality and Value-adding

2018, ARC, ARC-API, Westhuizen

‹ Lamb and Mutton Quality Audit

› Animal welfare, stress biomarkers and meat quality

DEADLINES for RESEARCHERS 2021

Proposals for 2021: TBC
Progress reports: 28 Jan 21
Final reports: 29 Jan 21 Final includes comprehensive report and popular article

COMMITEE MEETINGS for 2021

RMRDSA CSS Planning - TBC
Project Committee - TBC
Pork Planning - TBC

Calendar

< Apr 2021 >

Sun	Mon	Tue	Wed	Tur	Fri	Sat
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	

PORK Priority Areas

Cattle & Small Stock Programmes

1 Sustainable natural resource utilisation

2 Improvement of Livestock production and forage

3 Management of agricultural risk to create a resilient Red Meat sector

4 Sustainable health and welfare for the Red Meat sector

5 Enhancement of production and processing of Animal Products

6 Consumer and market development of the Red Meat sector

7 Commercialisation of the emerging sector