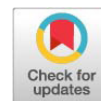


Review Article



Toll-Like Receptor Gene Polymorphisms and their Relationship with Performance and Immune Response in Sheep: A Systematic Review

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Abstract | The discovery of Toll-like receptors (TLRs) opened the door to a better understanding of the organism's defence mechanisms against infections. TLR family encodes several ancient immune receptors that recognize microbial conserved structural motifs termed as pathogen-associated molecular pattern (PAMPs). Studies that looked into genetic polymorphisms of TLR gene in sheep and their relationship with immune response have been conducted. Therefore, the objective of this systematic review was to systematically review the single nucleotide polymorphisms (SNPs) of the TLRs and their association with performance and immune response. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) were used for the identification, screening, eligibility and inclusion and 14 studies were found to be eligible. The systematic review used four search engines (Pubmed, Science direct, google scholar and web of science) and components of the research question identification of the Population, Exposure and Outcomes (PEO) to gather eligible studies. The results indicated that nucleotide sequences of 6 different TLRs were identified in the included articles. The results demonstrated that the identified TLRs (4 and 9) had a relationship with milk traits, flystrike response, susceptibility/resistance to Small Ruminant Lentivirus infections (SRLVs), pathogen detection, incidence of mastitis pathogens and immune response to pathogens. The TLRs influence the immune response of sheep, as such (1, 2, 4, 5, 7 & 9) TLRs have the potential to be useful genetic markers for improving performance and disease susceptibility in sheep during breeding programmes. Further research must investigate associations of the polymorphisms and plant-derived agonists that can activate the TLRs.

Keywords | Innate immunity, Polymorphism, Candidate gene, Pathogens, Sheep, Association

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INTRODUCTION

One of the most important processes in species evolution is host-pathogen competition (Smith *et al.*, 2014). Recent research in the field of natural resistance of animals and humans has increased the chances of controlling the pathogenic, metabolic and hereditary disease (Świderek *et al.*, 2006). Sheep breeds and individuals exhibit genetic variation in their susceptibility to infections, which may present a chance for genetic selection for resistance to infectious diseases by identifying the genes responsible

and investigating host-resistance mechanisms (Sarafidou *et al.*, 2013). The discovery of Toll-like receptors (TLRs) opened the door to a better understanding of the organism's defence mechanisms against infections (Świderek *et al.*, 2006).

Toll-like receptor (TLR) family encodes several ancient immune receptors that recognize microbial conserved structural motifs termed as pathogen-associated molecular pattern (PAMPs) (Yang *et al.*, 2018). Liu *et al.* (2021) reported that TLRs are a family of proteins that are

involved in the innate immune response and each TLR is able to identify patterns of molecules associated with pathogens, which can then trigger an innate immune response. TLR, a member of the interleukin-1 (IL-1) receptor superfamily, stimulates the immune system when microorganisms are present and enhances the immune system cells' ability to present antigens and kill microorganisms (Alan *et al.*, 2022). Additionally, they are divided into two subfamilies, the cell-surface TLRs and the intracellular TLRs, according to where they are located on or within the cell (Liu *et al.*, 2021). Among the TLRs, ten different types of TLRs are present in sheep (Chang *et al.*, 2009). TLRs 1, 2, 4–6, and 10, which are found on the cell surface, identify components of bacterial and other microbial cell walls, whereas TLRs 3, 7, 8, and 9, which are found intracellularly, particularly in endosomes, identify the nucleotide components of viruses and bacteria (Alan *et al.*, 2022).

Studies have shown that TLRs, which are a component of innate immunity, play a crucial role in host-pathogen interaction (Zhou *et al.*, 2006; Yaman, 2020; Liu *et al.*, 2021; Selala *et al.*, 2024). Several studies, Zhou and Hickford (2008), Smith *et al.* (2012), Yang *et al.* (2018), Yaman (2020), Arcangeli *et al.* (2021) and Liu *et al.* (2021) have investigated the genetic polymorphisms of TLR gene in sheep and their relationship with immune response. Although some research has examined the connection between TLR genes and immune response, there is no conclusive data to draw firm conclusions about how TLR genes affect immune response and production in sheep. As a result, a systematic review of the research findings will advance our understanding of TLRs as significant candidate genes for immune response and productive traits in sheep. Thus, this study will add knowledge on the use of TLRs as one of the candidate genes for performance traits and immune response in sheep.

MATERIALS AND METHODS

ELIGIBILITY CRITERIA

Prior to conducting the systematic review, the Population, Exposure, and Outcomes (PEO) components of the research question were identified as described by Bettany-Saltikov (2010). The population was described as “Sheep”, which was exposed to “Polymorphisms” and outcomes of “Immune responses/adaptive immunity”. Prior to the decision to conduct the systematic review, a preliminary search of the PEO components was conducted on the PubMed database.

LITERATURE SEARCH

The two authors (Thabang Sako and Nkululeko

Nyangiwe) used the databases of Google Scholar, PubMed, Science Direct, and Web of Science to search for research publications up until July 16, 2025, using the following keywords: ‘Toll-like receptors’, ‘polymorphisms/genetic variations’, ‘Immune response/adaptive immunity’, and ‘sheep’.

INCLUSION CRITERIA

All the obtained articles were deemed eligible if they met the following criteria: (1) the Toll-like receptor gene was being studied; (2) the polymorphisms of the Toll-like receptor gene were included; and (3) sheep were the species of interest.

EXCLUSION CRITERIA

The criteria for exclusion involved: (1) duplicated records; (2) lacking the association of TLRs polymorphisms to immune response/adaptive immunity; (3) and studies that inferred association without association analysis with immune response/adaptive immunity.

DATA EXTRACTION

The content that was extracted was done independently by the two authors (Thabang Sako and Nkululeko Nyangiwe). The contents extracted from the articles include the name of the 1st author, the year the article was published, country, breed, population size, Toll-like receptor gene number and observed genotypes.

RESULTS

SEARCH RESULTS

A total of 165 articles were retrieved for the systematic review from the following databases: Google Scholar (n = 57), PubMed (n = 38), Science Direct (n = 19), and Web of Science (n = 51). After removing the duplicates (n = 82) that appeared between the search databases, the remaining articles underwent additional analysis for inclusion and exclusion criteria. The remaining articles (n = 83) were screened for their title, and 57 (n = 57) were eliminated. Next, the abstract was screened, and nine (n = 9) were eliminated. Three (n = 3) articles were eliminated from the entire article search, leaving a total of eighteen (n = 14) articles to be included in the systematic review.

CHARACTERISTICS OF INCLUDED STUDIES

A total number of fourteen articles (n = 14) of the hundred and sixty-five analysed were kept and incorporated in the systematic review as shown in Table 1. Each author had one (n = 1) each of the 14 included articles except for Smith (Smith *et al.*, 2012, 2014) Yaman (Yaman, 2020; Yaman *et al.*, 2021) and Zhou (Zhou *et al.*, 2006; Zhou and Hickford, 2008) who had two (n = 2) articles each in the 14 included articles. The used articles ranged from 2006 to 2024. The

articles were from nine differing countries. The sample size of sheep utilised ranged from 50-1 750, with 28.57%, (n= 4) of the studies ranging from 50-150 sheep used. The included studies investigate the toll-like receptors (TLRs) and their association with traits in sheep with the Merino sheep probing most, being investigated in (n= 4) studies

which is 28.57% of included articles. The included studies investigated six different TLRs (1, 2, 4, 5, 7 and 9), with TLR4, 50%, being the most investigate TLR. Polymerase chain reaction- single strand conformation polymorphism (PCR-SSCP) was the most used genotyping method, with 50% of the 14 articles that were included.

Table 1: General characteristics of studies included in the review.

Author	Year	Country	Breed	N	Sex	Gene	Trait	Genotypic method
Arcangeli <i>et al.</i> ,	2021	Italy	Italian sheep	493	N/A	TLR 9	SRLV infection	PCR qPCR
Byun <i>et al.</i> ,	2009	New Zealand	N/A	1 670	N/A	TLR 4	Immune response, Disease susceptibility	PCR-SSCP
Liu <i>et al.</i> ,	2021	New Zealand	Merino, Corriedale, Perendale, Romney, Lincoln, Coopworh, Poll Dorset, Texel, Finn, Dorset Down, Suffolk, South Suffolk, Shropshire, and various crossbred sheep and composites)	212	N/A	TLR 9	Flystrike susceptibility	PCR-SSCP
Sallam,	2021	Egypt	Barki	311	Females	TLR 4	Milk traits	PCR-SSCP
Sarafidou <i>et al.</i> ,	2013	Greece	Boutsko-breed (n=56), Friesarta-breed (n=27) and Comisana breed (n=32)	115	N/A	TLR 9	SRLV	
Selala <i>et al.</i> ,	2024	South Africa	Dorper	50	N/A	TLR 4		PCR
Smith <i>et al.</i> ,	2012	England	Blackface, Blueface, Djallonke, Dorper, Dorset, Latxa, Red Maasai, Suffolk, Texel, Soay	87	N/A	TLR 5		
Smith <i>et al.</i> ,	2014	England	Blackface, Blueface, Djallonke, Dorper, Dorset, Latxa, Red Maasai, Suffolk, Texel, Soay	69	N/A	TLR 2	Innate immunity	PCR
Świderek <i>et al.</i> ,	2006	Poland	Polish Heath sheep (n=130) and Polish Lowland sheep (n =130)	260	N/A	TLR 1, 2 and 4	Somatic cell concentration and natural bacterial infections of the mammary gland	PCR-SSCP
Yaman,	2020	Türkiye	Karacabey merinosu, Kıvrıcık, İmroz, Bandırma, Ramlıç, Black head mutton merino crossbred-SBA, and Hampshire crossbred	1 750	Females	TLR 4	Theovine Johne's disease	PCR
Yaman <i>et al.</i> ,	2020	Türkiye	Karacabey merino (n=67), Kivircik (n=18), Imroz (n=38), Chios (Sakiz) (n=18), Cine capari (n=43) and Karak-acan (n=41)	225	N/A	TLR 4	presence of <i>Eimeria</i> spp.	PCR
Yang <i>et al.</i> ,	2018	China	Suffolk (n=600) and Kazak sheep (n=600)	1 200	N/A	TLR 7	Susceptibility to diseases	PCR SSCP
Zhou and Hickford,	2008	New Zealand	New Zealand Romney sheep	520	N/A	TLR 9	Disease resistance	PCR SSCP
Zhou <i>et al.</i> ,	2006	New Zealand	Merino, Corriedale, Romney, Poll Dorset, and NZ cross-bred sheep	412	N/A	TLR 4	Immune response to pathogen	PCR-SSCP

N/A: not available; N: sample size; SRLVs: small ruminant lentiviruses; SRLV: small ruminant lentivirus; TLR1: toll-like receptor 1; TLR2: toll-like receptor 2; TLR4: toll-like receptor 4; TLR5: toll-like receptor 5; TLR7: toll-like receptor 7; TLR9: toll-like receptor 9; PCR – polymerase chain reaction, SSCP: single strand conformation polymorphism, RFLP: restriction fragment length polymorphism.

The 14 included studies' publication years are shown in Figure 2. The results demonstrate that the included studies came from 2006-2024. Out of 14 articles, the majority were published in 2021 with three articles out of 14 (Arcangeli *et al.*, 2021; Liu *et al.*, 2021; Sallam, 2021) 2 years (2006 and 2020) included two studies each and seven years (2008, 2009, 2012, 2013, 2014, 2018, and 2024) included one study each out of 14.

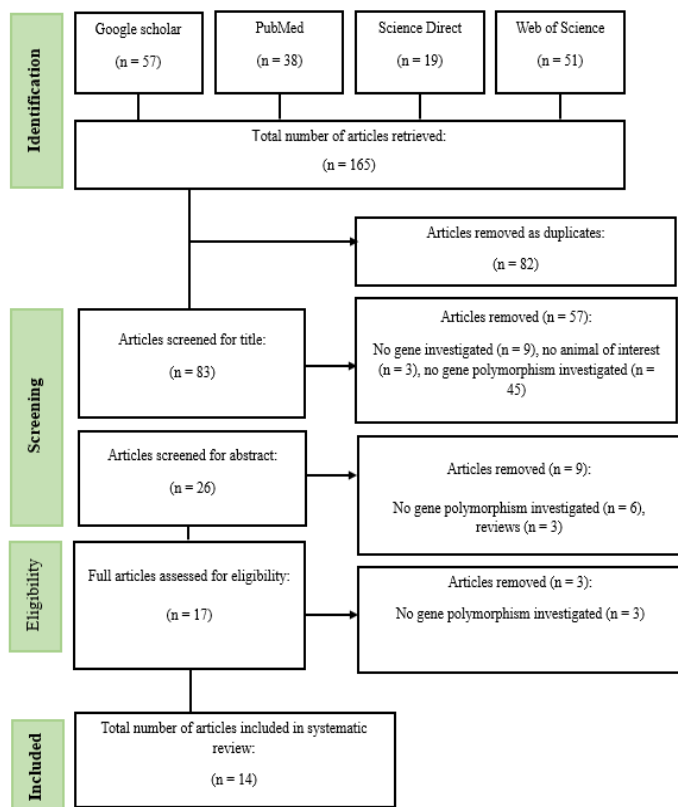


Figure 1: Study selection flow diagram.

PUBLICATION BY COUNTRY

The included studies' country of publication is shown in Figure 3. The findings indicate that the fourteen included studies were carried out in nine differing countries. There were four articles in New Zealand (Zhou *et al.*, 2006; Zhou and Hickford, 2008; Byun *et al.*, 2009; Liu *et al.*, 2021), 2 countries (England and Türkiye) had two articles each and 6 countries (Italy, Egypt, Greece, South Africa, Poland and China) had one study each.

DISTRIBUTION BY JOURNAL

The distribution of included studies by journal is shown in Figure 4. The results showed that they were published in thirteen different international journals including viruses, veterinary immunology and immunopathology, animals, animal bioscience, plos one, journal of advanced veterinary and animal research, BMC evolutionary biology, gene, folia microbiologica, Turkish journal of veterinary and animal sciences, animal biotechnology,

Indian journal of animal research, and developmental and comparative immunology. Veterinary immunology and immunopathology contributed three journals (Zhou and Hickford, 2008; Byun *et al.*, 2009), while the rest of the journals contributed each on the other 14 included articles.

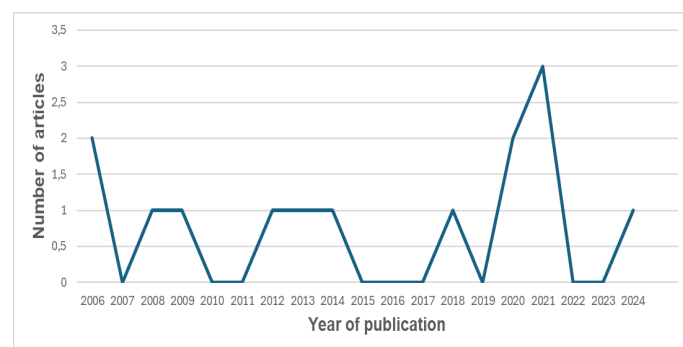


Figure 2: Publication by year.

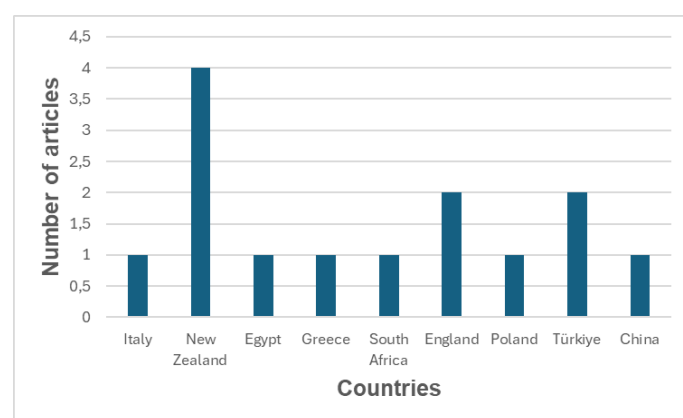


Figure 3: Distribution by country.

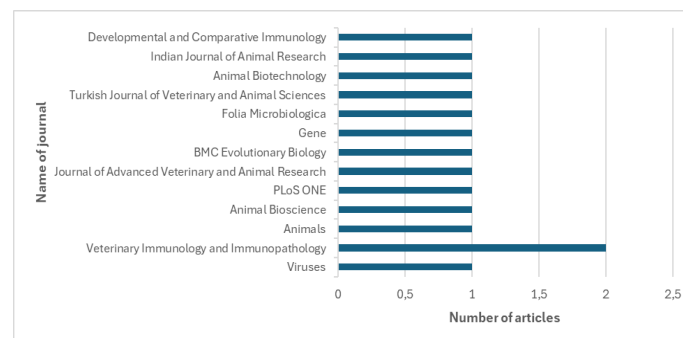


Figure 4: Publication by journal.

IDENTIFIED SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) AND TARGETED GENOMIC REGION

Table 2 displays the identified SNPs along with the genomic regions of the TLRs under investigation. Nine of the fourteen included articles identified the SNPs and their targeted regions, according to the findings. The findings identified a total of 18 SNPs for TLR2, 23 SNPs for TLR4, 25 SNPs for TLR5, 5 SNPs for TLR7 and 8 SNPs for TLR9. The results indicated that the TLR4 SNPs were found on exon 2 and 3 while TLR9 SNPs were found on exon 2. 8 articles (57.14%) out of the 14 includes articles

indicated the genomic regions of the identified SNPs.

GENOTYPIC FREQUENCIES

The reported genotypic frequencies of the include studies are shown in Table 3. A total of 21 discovered genotypes were reported throughout the study. The results indicated that the TLR4 reported in the two studies showed 2 and 3 genotypes, respectively. TLR9 reported 3 genotypes while TLR7 reported 3 genotypes and 2 genotypes. Yang *et al.* (2018) reported the highest genotypic frequency (0.942) and the lowest genotypic frequency (0.058).

ALLELIC FREQUENCIES

The allelic frequencies of the included studies are presented in Table 4. A total of 50 discovered alleles were reported throughout the study. The results showed that 6 out of 14 included articles were clear about the allelic frequencies, while 8 were not. The reported alleles for TLR1, TLR2, TLR4 and TLR9 were 4, 10, 30 and 6, respectively. The highest (0.973) and lowest (0.023) allelic frequencies were reported by Świderek *et al.* (2006).

Table 2: SNPs and genomic regions.

Author	Breed	TLR	Region	SNP
Arcangeli <i>et al.</i> , 2021	Italian sheep	TLR9	N/A	R447Q, A462S, G520R
Byun <i>et al.</i> , 2009	N/A	TLR4	Exon 3	T955C, T1013C, G1032C, A1045G, G1052A, T1066C, A1088G, T1091C, C1097G, C1132T, G1166C, G1183T
Liu <i>et al.</i> , 2021	Merino, Corriedale, Perendale, Romney, Lincoln, Coopworth, Poll Dorset, Texel, Finn, Dorset Down, Suffolk, South Suffolk, Shropshire, and various crossbred sheep and composites	TLR9	Exon 2	c.1323T/C, c.1340G/A (p.Arg447Gln), C.1384G/T (p.Ala462Ser), c.1452G/A, c.1558C/G (p.Arg520Gly), c.1572C/T
Sallam, 2021	Barki	TLR4	Exon 3	c.1710C>A
Sarafidou <i>et al.</i> , 2013	Boutsko-breed, Friesarta-breed and Comisana breed	TLR9	N/A	C1323T, G1340A, G1384T, G1557C, T1571C
Selala <i>et al.</i> , 2024	Dorper	TLR4	Exon 2	T2249C
Smith <i>et al.</i> , 2012	Blackface, Blueface, Djallonke, Dorper, Dorset, Latxa, Red Maasai, Suffolk, Texel, Soay	TLR5	N/A	G33G, C46A, G210G, C275C, G339G, C360C, T396T, A731A, T906T, C1200C, A1474G, C1531A, A1536A, G1698G, T1887T, A1953A, A1976A, A1977A, A2016A, A2047G, T2157T, A2276A, G2353C, T2394T, A2418A
Smith <i>et al.</i> , 2014	Blackface, Blueface, Djallonke, Dorper, Dorset, Latxa, Red Maasai, Suffolk, Texel, Soay	TLR2	N/A	C615C, G674G, C943C, G1162T, A1401A, C1688C, G1731G, C1737C, T1749T, C1949C, T2008C, T2037T, A2055A, C2089T, C2168C, G2232G, G2235G, C2238C
Świderek <i>et al.</i> , 2006	Polish Heath sheep and Polish Lowland sheep	TLR1 TLR2 TLR4	N/A	N/A
Yaman, 2020	Karacabey merinosu, Kivircik, İmroz, Bandırma, Ramlıç, Black head mutton merino crossbred-SBA, and Hampshire crossbred	TLR4	Exon 3	G688A, A857G, G873A, G881A, A883G, T892C, C897T, G1032C, G1052A, T1066C, A1088G, T1091C, C1097G, C1132T, G1183T
Yaman <i>et al.</i> , 2020	Karacabey merino, Kivircik, Imroz (Gokceada), Chios (Sakiz), Cine capari and Karakacan	TLR4	Exon 3	A857G, G873A, G881A, A883G, T892C, C897T, T1066C, A1088G, T1091C, C1097G, C1132T, G1183T, G1540A, G2037A
Yang <i>et al.</i> , 2018	Suffolk and Kazak sheep	TLR7	N/A	A343G, A350G, G864A, T1185C, A1244G
Zhou and Hickford, 2008	New Zealand Romney sheep	TLR9	Exon 2	C1323T, G1340A, G1384T, G1452A
Zhou <i>et al.</i> , 2006	Merino, Corriedale, Romney, Poll Dorset, and NZ cross-bred sheep	TLR4	Exon 3	A857G, G873A, G881A, A883G, T892C, C897T, G1032C, G1052A, T1066C, A1088G, T1091C, C1097G, C1132T, G1183T

N/A: not available; N: sample size; TLR1: toll-like receptor 1; TLR2: toll-like receptor 2; TLR4: toll-like receptor 4; TLR5: toll-like receptor 5; TLR7: toll-like receptor 7; TLR9: toll-like receptor 9.

Table 3: Genotypic frequencies.

Author	Breed	N	TLR	Genotypic frequency
Sallam <i>et al.</i> , 2021	Barki	311	TLR4	GG (0.446), GT (0.357), TT (0.197)
Sarafidou <i>et al.</i> , 2013	Boutsko-breed, Friesarta-breed and Comisana breed	115	TLR9	RR (0.091), GG (0.197), GR (0.712) RR (0.392), GG (0.113), GR (0.495) RR (0.414), GG (0.131), GR (0.455)
Selala <i>et al.</i> , 2024	Dorper	50	TLR4	TT (0.60), TC (0.40)
Yang <i>et al.</i> , 2018	Suffolk and Kazak sheep	1 200	TLR7	AA (0.55), AB (0.33), BB (0.12) GG (0.942), HH (0.058) AA (0.63), BB (0.37)

N: sample size; TLR4: toll-like receptor 4; TLR7: toll-like receptor 7; TLR9: toll-like receptor

Table 4: Allelic frequencies.

Author	Breed	N	TLR	Allelic frequencies
Byun <i>et al.</i> , 2009	N/A	1 670	TLR4	01 (0.471), 02 (0.358), 03 (0.115), 04 (0.047), 05 (0.003), 06 (0.003), 07 (0.003)
Sallam <i>et al.</i> , 2021	Barki	311	TLR4	G (0.635), T (0.365)
Sarafidou <i>et al.</i> , 2013	Boutsko-breed, Friesarta-breed and Comisana breed	115	TLR9	R (0.447), G (0.553) R (0.64), G (0.36) R (0.64), G (0.36)
Selala <i>et al.</i> , 2024	Dorper	50	TLR4	T (0.80), C (0.20)
Świderek <i>et al.</i> , 2006	Polish Heath sheep and Polish Low-land sheep	130 + 130	TLR1	01 (0.877) 01 (0.973) 02 (0.123) 02 (0.023)
			TLR2	01 (0.692) 01 (0.837) 02 (0.1080) 02 (0.139) 03 (0.023) 04 (0.054) 05 (0.077) 05 (0.016) 06 (0.046) 06 (0.008)
			TLR4	01 (0.285) 01 (0.239) 02 (0.123) 02 (0.308) 03 (0.154) 03 (0.231) 04 (0.038) 04 (0.169) 05 (0.115) 05 (0.038) 06 (0.123) 07 (0.085) 09 (0.023) 10 (0.054) 10 (0.015)
Zhou <i>et al.</i> , 2006	Merino, Corriedale, Romney, Poll Dorset, and NZ cross-bred sheep	412	TLR4	01 (0.57), 02 (0.18), 03 (0.12), 04 (0.13)

N/A: not available; N: sample size; TLR1: toll-like receptor 1; TLR2: toll-like receptor 2; TLR4: toll-like receptor 4; TLR9: toll-like receptor 9.

SINGLE NUCLEOTIDE POLYMORPHISMS AND THEIR ASSOCIATIONS WITH SHEEP PRODUCTION TRAITS

The SNPs and their associations with production traits and immune response are shown in Table 5. The results showed that the 14 included studies studied immune responses to various infections and milk production traits and their associations with the genotypes of identified SNPs of the TLRs. The results indicated a significant relationship between the genotypes of TLR9 SNPs and the small ruminant lentiviruses infection. No significant relationship we indicated between the infections of *m. avium subsp. Paratuberculosis* and *C. abortus* and genotypes

of TLR9 SNPs. The genotypes of the SNPs of TLR4 showed a significant relationship with 2 milk traits (fat percentage and protein percentage) and non-significant relationship with 2 milk traits (total solids percentage and daily milk yield).

DISCUSSION

Infectious diseases have a negative impact on sheep health and productivity (Sarafidou *et al.*, 2013). In addition to triggering the adaptive immune response, innate immune mechanisms offer a first line of defense against infection by

Table 5: Single nucleotide polymorphisms and their associations with immune response and sheep production traits as reported by original studies.

Author	Breed	TLR	Traits	Genotypes	Significance
Arcangeli <i>et al.</i> , 2021	Italian sheep	TLR9	SRLVs Infection	QQ RQ RR	*
				AA AS SS	*
				RR GR GG	*
Sallam <i>et al.</i> , 2021	Barki	TLR4	DMY	TT GT GG	ns
			FP	TT GT GG	*
			PP	TT GT GG	*
			LP	TT GT GG	ns
			TSP	TT GT GG	ns
Sarafidou <i>et al.</i> , 2013	Boutsko-breed, Friesarta-breed and Comisana breed	TLR9	SRLV	RR GG GR	*
			CA	RR GG GR	ns
			MAP	RR GG GR	ns
Yaman <i>et al.</i> , 2020	Karacabey merino, Kivircik, Imroz, Chios, Cine capari and Karakacan	TRL4	<i>Eimeria</i> spp.	N/A	*
				N/A	ns

TLR4: toll-like receptor 4; TLR9: toll-like receptor 9; SRLVs: small ruminant lentiviruses; DMY: daily milk yield (g/d); FP: fat percentage; PP: protein percentage; LP: lactose percentage; TSP: total solids percentage; CA: *C. abortus*; MAP: *m. avium* subsp. *Paratuberculosis*; * Significance estimated at $p < 0.05$; ns: non-significant $p > 0.05$; N/A; not available.

identifying and eliminating pathogens (Zhou *et al.*, 2006). According to Liu *et al.* (2021), a family of proteins known as toll-like receptors (TLRs) is involved in the innate immune response. This systematic review was conducted to establish the impact of TLRs on the performance and immune response in sheep.

The results of this systematic review indicated that out of the 14 included studies, there were 79 different Single Nucleotide Polymorphisms (SNPs) found with the Exon 3 Being the most targeted genomic region. Arcangeli *et al.* (2021) investigated the TLR4 in Italian sheep and identified three SNPs (R447Q, A462S and G520R) that had a relationship with the small ruminant lentiviruses. Byun *et al.* (2009) investigated the TLR4 in sheep and identified 12 SNPs (T955C, T1013C, G1032C, A1045G, G1052A, T1066C, A1088G, T1091C, C1097G, C1132T, G1166C and G1183T) at exon 4. Sallam (2021) investigated TLR4 and discovered a c.1710C>A SNP an association between the TLR4 and fat percentage or protein percentage, while there was no association between the TLR and daily milk yield or total solids percentage reported in Barki ewes. Sarafidou *et al.* (2013) reported associations between the G520C SNP and small ruminant lentiviruses while there were no associations between the SNP and *M. avium* subsp. *Paratuberculosis* or *C. abortus* in the three breeds (Friesarta-breed, Boutsko-breed and Comisana breed) that were investigated.

Study by Selala *et al.* (2024) on TLR4 in Dorper sheep identified a SNP (T2249) at Exon 2. Smith *et al.* (2012) investigated the evolution of TLR5 in domestic animals

and detected twenty-five SNPs (G33G, C46A, G210G, C275C, G339G, C360C, T396T, A731A, T906T, C1200C, A1474G, C1531A, A1536A, G1698G, T1887T, A1953A, A1976A, A1977A, A2016A, A2047G, T2157T, A2276A, G2353C, T2394T and A2418A) in Blackface, Texel, Blueface, Dorper, Djallonke, Dorset, Red Maasai, Latxa, Suffolk, Soay sheep. The study of Smith *et al.* (2014) investigating the novel ovine polymorphisms and adaptive evolution in mammalian TLR2 suggest existence of multiple pathogen binding regions identified 18 SNPs (C615C, G674G, C943C, G1162T, A1401A, C1688C, G1731G, C1737C, T1749T, C1949C, T2008C, T2037T, A2055A, C2089T, C2168C, G2232G, G2235G and C2238C) spread across the Blackface, Blueface, Djallonke, Dorper, Dorset, Latxa, Red Maasai, Suffolk, Texel and Soay sheep breeds. Świderek *et al.* (2006) investigated TLRs and their associations with the natural bacterial infections of the mammary gland and somatic cell concentrations in sheep and detected two alleles of TLR1, six alleles of TLR2 and nine alleles of TLR4 in the Polish Heath sheep ewes and detected two alleles of TRL1, four alleles of TLR2 and six alleles of TLR4 in Polish Lowland sheep ewes. Yaman (2020) investigated TLR4 in three Turkish native (Kivircik, Karacabey merinosu and Imroz) and 4 composite (Ramlic, Bandirma, Hampshire crossbred and Black head mutton merino crossbred-SBA) and identified 15 SNPs (G688A, A857G, G873A, G881A, A883G, T892C, C897T, G1032C, G1052A, T1066C, A1088G, T1091C, C1097G, C1132T and G1183T) on exon 3 and reported that there were no associations detected between the investigated TLR4 and paratuberculosis in composite sheep and Turkish native sheep. The study by

[17] investigated TLR4 in six indigenous Turkish sheep breeds (Kivircik, Karacabey merino, Chios (Sakiz), Imroz (Gokceada), Cine capari and Karakacan) and identified 14 SNPs (A857G, G873A, G881A, A883G, T892C, C897T, T1066C, A1088G, T1091C, C1097G, C1132T, G1183T, G1540A and G2037A) of Exon 3 and a relationship between SNP T1066C and *Eimeria* spp. infection (coccidiosis) was detected.

Yang *et al.* (2018) examined the polymorphisms of TLR7 in Suffolk and Kazak sheep and identified three SNPs (A343G, A350G and G864A) in Kazak sheep and five SNPs (A343G, A350G, G864A, T1185C and A1244G) in Suffolk sheep. Therefore, two SNPs (T1185C and A1244G) were identified in Suffolk and not found in Kazak sheep. Zhou and Hickford (2008) investigated the variation of the TLR9 gene in sheep and identified four SNPs (C1323T, G1340A, G1384T and G1452A) in the New Zealand Romney sheep. Zhou *et al.* (2006) investigated the allelic variation of the TLR4 gene in sheep and identified fourteen SNPs (A857G, G873A, G881A, A883G, T892C, C897T, G1032C, G1052A, T1066C, A1088G, T1091C, C1097G, C1132T and G1183T) in the five sheep breeds (Merino, Romney, Corriedale, NZ cross-bred sheep and Poll Dorset).

The contribution of this systematic review to the body of knowledge is that there were eighteen (C615C, G674G, C943C, G1162T, A1401A, C1688C, G1731G, C1737C, T1749T, C1949C, T2008C, T2037T, A2055A, C2089T, C2168C, G2232G, G2235G and C2238C) identified SNPs for TLR2, twenty-three (G688A, A857G, G873A, G881A, A883G, T892C, C897T, T955C, T1013C, G1032C, A1045G, G1052A, T1066C, A1088G, T1091C, C1097G, C1132T, G1166C, G1183T, G1540A, c.1710C>A, G2037A and T2249C) Identified SNPs for TLR4, twenty-five (G33G, C46A, G210G, C275C, G339G, C360C, T396T, A731A, T906T, C1200C, A1474G, C1531A, A1536A, G1698G, T1887T, A1953A, A1976A, A1977A, A2016A, A2047G, T2157T, A2276A, G2353C, T2394T and A2418A) identified SNPs for TLR5, five (A343G, A350G, G864A, T1185C and A1244G) identified SNPs for TLR7 and eight (C1323T, G1340A, C.1384G/T (p.Ala462Ser), G1452A, G1557C, c.1558C/G (p.Arg520Gly), T1571C and c.1572C/T) identified SNPs for TLR9. TLR4 SNPs were found on exon 3 and TLR9 SNPs were found on exon 2. Based on our knowledge, this is the first systematic review reporting on the association between SNPs of the TLRs in sheep and immune response or production in sheep. Therefore, there were no comparisons of the other systematic review in this field. The limitations of this systematic review are that (a) some articles identified SNPs and their relationship with immune response was not disclosed and (b) identified

SNPs were not similar in the included studies which make is not feasible to conduct a meta-analysis.

CONCLUSIONS AND RECOMMENDATIONS

The TRLs influence the immune response and production in sheep and as such, there were identified SNPs of TLR1, TLR2, TLR4, TLR5, TLR7 and TLR9. The SNPs of the TLR9 improvement would improve the resistance of sheep to SRLVs infections and might be used as potentially viable genetic indicators for the betterment of immune response and c.1710C>A SNPs of TLR4 might be used for the betterment of milk production in sheep during breeding programs.

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NOVELTY STATEMENT

This study offers valuable information on how TLRs can be used as candidate genes for production traits and immune response in sheep. The findings provide important insights on sheep selection and breeding for improved performance and immune response.

AUTHOR'S CONTRIBUTION

This manuscript was written with input from all authors. Thabang Sako wrote the manuscript's initial draft. Mpho Tawana, Nkululeko Nyangiwe, and Ntombikhona Koza then made revisions to the manuscript. Every author reviewed and approved the final manuscript.

ETHICAL CONSIDERATIONS

All authors took into account ethical concerns such as plagiarism, misconduct, informed consent, fabrication, and data falsification.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that this manuscript was prepared without the use of Generative AI.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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