Protocol for a Systematic Review on Candidate Gene Polymorphisms Associated with Newcastle Disease Outcomes in Chickens

Authors and their affiliations

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Author contributions

The review (PICO) question and the protocol described in this document were developed with the contribution and final approval of all co-authors. Nsiyapnze Katte Yato Katte drafted the protocol, and all authors provided their input.

Registration

This protocol is archived at the University of Dschang Institutional Repository and will be registered with Systematic Reviews for Animals and Food (SYREAF) available at: http://www.syreaf.org/. This protocol is reported using the items (headings) recommended in the PRISMA-P guidelines (Shamseer *et al*., 2015).

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1. Introduction

1.1. Rationale

Newcastle disease (ND) is a highly contagious and economically devastating viral disease of chickens caused by avian orthoavulavirus 1 (AOAV-1; formerly APMV-1/NDV), responsible for catastrophic mortality in susceptible flocks globally. In domestic chickens (*Gallus gallus domesticus*), ND ranks among the most economically devastating diseases

in poultry production, with velogenic strains capable of causing flock mortality up to 100%. Clinical manifestations encompass respiratory, neurological, and enteric disorders, resulting in substantial production losses across both commercial and smallholder poultry operations globally (Ganar *et al*., 2014).

Current ND control relies principally on vaccination programmes and biosecurity measures. However, vaccine efficacy can be undermined by antigenic divergence in circulating NDV strains, maternal antibody interference, heat lability of live vaccines in resource-limited settings, and immunosuppression. In particular, for subsistence poultry keepers in Africa and Asia, where vaccine cold chains are unreliable, alternative disease management tools are critically needed. Genetic selection for enhanced host resistance represents a sustainable, infrastructure-independent, and cumulative strategy that can complement vaccination.

The genetic architecture of Newcastle disease resistance in chickens is complex and polygenic, engaging multiple pathways of innate and adaptive immunity. Three candidate genes have attracted the greatest research attention: the myxovirus resistance (Mx) gene, the major histocompatibility complex (MHC/B complex), and the roundabout guidance receptor 2 (ROBO2) gene. The Mx gene encodes a dynamin-like GTPase that is upregulated by type I and type III interferons and exerts antiviral activity by interfering with viral nucleocapsid assembly and intracellular trafficking. A G/A SNP in the chicken Mx gene has been associated with differential antiviral potency against NDV and other viruses across multiple populations. The chicken MHC, located on chromosome 16, encompasses class I (B-F) and class II (B-L) genes whose products present viral antigens to T lymphocytes, thereby initiating adaptive immune responses. The B complex exhibits extensive polymorphism; specific haplotypes and alleles influence antibody responses following NDV vaccination and susceptibility to NDV challenge. The microsatellite marker LEI0258, in strong linkage disequilibrium with the MHC-B region, is widely employed as a proxy for MHC haplotyping in field studies. ROBO2, originally characterised for axonal guidance in neural development, is a member of the immunoglobulin superfamily with emerging evidence for roles in immune cell trafficking and activation.

Beyond these three genes, unbiased genome-wide approaches employing GWAS and QTL mapping have identified additional loci and candidate genes, including TIRAP, ETS1, CAMK1d, and KIRREL3, involved in Toll-like receptor signalling, transcriptional regulation of immune genes, and lymphocyte adhesion, respectively. Despite the growing body of evidence, a systematic synthesis of the fragmented literature is urgently needed to identify reproducible associations, evaluate methodological quality, and guide future research and breeding programme design.

This systematic review aims to comprehensively evaluate and synthesise the evidence on associations between candidate gene polymorphisms and Newcastle disease outcomes in

chickens. The review will quantify the current state of genetic resistance markers, identify predominant resistance-conferring alleles and their antimicrobial resistance implications, highlight population-specific effects across different chicken breeds and geographic regions, and inform evidence-based interventions for genetic improvement programmes. The findings will guide future research priorities and genomic selection strategies in poultry breeding.

1.2. Objectives

This protocol aims to systematically review and synthesise the available evidence on associations between candidate gene polymorphisms and Newcastle disease outcomes in chickens, providing a comprehensive assessment of genetic resistance patterns and their implications for marker-assisted and genomic selection programmes.

- Population: Domestic chickens (*Gallus gallus domesticus*) as the primary species

- Interest: Polymorphisms, variants, or haplotypes in candidate genes (Mx, MHC/B complex including B-F and B-LB genes and LEI0258 microsatellite, ROBO2, or other molecularly characterised loci identified through GWAS or QTL mapping)

- Context: Poultry production systems (backyard, semi-intensive, intensive) globally, with emphasis on Africa, Asia, North America, and Europe

2. Methods

2.1. Eligibility Criteria

Criteria related to the elements of the PICOS question:

Population: Domestic chickens (*Gallus gallus domesticus*) as the primary species, encompassing commercial lines, indigenous/local breeds, inbred MHC-defined lines, and crossbreds.

Exposure: Naturally occurring or experimentally induced polymorphisms, SNPs, haplotypes, or microsatellite variants in candidate genes: Mx, MHC (B-F, B-LB, LEI0258), ROBO2, or other molecularly characterised loci identified through GWAS or QTL approaches.

Comparator: Different genotypes, alleles, or haplotypes at the same locus; contrasts between homozygous and heterozygous individuals or between defined allele-carrying and non-carrying groups.

Outcome: Newcastle disease-related phenotypes: antibody titres (HI or ELISA), viral load/titre, viral shedding, clinical scores, mortality/survival, T cell proliferation/activation, body weight change post-infection.

Study design: Candidate gene association studies, GWAS, QTL mapping, controlled challenge trials with genotyping, and functional studies (knockout/overexpression);

narrative reviews, editorials, conference abstracts, and expression-only studies (without polymorphism stratification) will be excluded.

Publication types: Journal articles that provide results of original research and fulfil the study design eligibility criteria.

Language: Research articles published in English or French.

Publication date: January 2000 to April 2026.

Geographical location: No geographical restrictions; studies from Africa, Asia, North America, Europe, and other regions will be included.

Inclusion criteria:

- Target population: Domestic chickens (*Gallus gallus domesticus*) as the primary species.
- Specified genetic markers: Investigation of polymorphisms, variants, or haplotypes in Mx, MHC (including B-F, B-LB, LEI0258), ROBO2, or other molecularly characterised candidate genes.
- Newcastle disease phenotypes: Measurement of specific NDV-related outcomes (survival, viral load/shedding, clinical signs, antibody responses).
- Genotype–phenotype association: Explicit linkage between genetic variants and measured ND outcomes.
- Acceptable study designs: GWAS, QTL mapping, candidate gene association studies, controlled challenge trials with genotyping, and functional studies (knockout/overexpression).

Exclusion criteria:

- Expression-only data: Studies reporting gene expression (mRNA/transcriptomics) without stratification by specific genetic polymorphisms.
- Other avian diseases: Studies focused on genetic resistance to other poultry diseases (e.g., Marek's disease, avian influenza) unless they also reported specific ND outcome data.
- Lack of genetic variation comparison: Studies evaluating ND responses in different chicken lines without identifying specific polymorphic markers.
- Insufficient study type: Narrative reviews, conference abstracts without full methods/results, editorials, pure polymorphism surveys without NDV phenotype data, and studies where the primary pathogen was not NDV.

2.2. Information Sources

The search will be conducted using bibliographic databases that offer comprehensive coverage of biomedical, veterinary, and agricultural literature. The list of databases to be searched is reported in Table 1.

Table 1. Databases used for the Systematic Literature Review

Database	Interface	URL
MEDLINE	PubMed	https://pubmed.ncbi.nlm.nih.gov/
Web of Science	Web of Science	https://www.webofscience.com/
SCOPUS	Elsevier	https://www.scopus.com
Google Scholar	Google Scholar	https://scholar.google.com/
arXiv	arXiv	https://arxiv.org/

Additional sources: Reference lists of included studies will be hand-searched, and citation tracking of key papers will be performed. Authors will be contacted for unpublished data or supplementary information where necessary.

2.3. Search Strategy

The search strategy will employ a multi-strand approach (Higgins et al., 2021). The search strategy will be tailored to each database to ensure thorough coverage of relevant literature. If fewer than 10 papers are included, a backwards search using Scopus or Web of Science databases will be performed.

The following search strategy will be used:

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#1 (poultry OR chicken* OR chick* OR broiler* OR layer* OR turkey* OR duck* OR fowl* OR avian* OR "Gallus gallus")
#2 ("Newcastle disease" OR "Newcastle disease virus" OR NDV OR "avian orthoavulavirus 1" OR APMV-1 OR AOAV-1)
#3 ("genetic resistance" OR "candidate gene*" OR "Mx gene" OR Mx OR "major histocompatibility complex" OR MHC OR "B complex" OR "B-F" OR "B-LB" OR "LEI0258" OR ROBO2 OR "roundabout guidance receptor" OR GWAS OR "genome-wide association" OR QTL OR "quantitative trait locus" OR polymorphism* OR SNP OR haplotype* OR allele* OR genotype*)
#4 (antibody OR "antibody titre" OR "antibody titer" OR "viral load" OR "viral shedding" OR survival OR mortality OR "clinical score" OR "T cell" OR "immune response" OR "body weight")
#5 #1 AND #2 AND #3 AND #4
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Analogous queries, substituting MHC/B complex and ROBO2 as the target gene terms, will be equally used. Complete query strings for all databases are available from the corresponding author upon request.

2.4. Study Record Data Management

For the purpose of deduplication, all recovered records will be loaded into Zotero Software. The file will be transferred to the Rayyan program for screening once duplicates have been eliminated.

2.4.1 Selection Process

There will be two stages involved in screening the retrieved citations: i) screening of the titles and abstracts, and ii) screening of the entire content.

Screening will be done by independent reviewers. In order to guarantee that every reference is examined by a minimum of two independent reviewers, each group will be given half of the citations. Any disagreements will be settled by discussion or by seeking advice from a third reviewer. Each screening phase will start by a calibration exercise based on at least 5% of randomly selected articles. This will help to facilitate discussion and resolve conflict prior to executing the entire selection process (Shamseer et al., 2015).

For title and abstract screening, the eligibility of the studies will be assessed using the following questions:

- Is the study original research in English or French? Yes [include], No [exclude], unclear [include]
- Does the study focus on domestic chickens (Gallus gallus domesticus)? Yes [include], No [exclude], unclear [include]
- Does the study investigate genetic polymorphisms or variants in candidate genes? Yes [include], No [exclude], unclear [include]
- Does the study measure Newcastle disease virus (NDV) outcomes? Yes [include], No [exclude], unclear [include]

Records meeting the inclusion criteria will proceed to the next stage. During the full text screening, the eligibility of the studies will be assessed using the following questions:

- Is the full text available in English or French? Yes [include], No [exclude]
- Is the study original research? Yes [include], No [exclude]
- Is the population of study domestic chickens (Gallus gallus domesticus)? Yes [include], No [exclude], unclear [exclude]
- Does the study specifically investigate polymorphisms in Mx, MHC (B-F, B-LB, LEI0258), ROBO2, or other GWAS/QTL-identified candidate genes? Yes [include], No [exclude], unclear [exclude]

- Does the study measure specific NDV-related outcomes (antibody titres, viral load, clinical scores, mortality, immune responses, viral shedding, body weight change)? Yes [include], No [exclude], unclear [exclude]
- Does the study report explicit genotype–phenotype associations? Yes [include], No [exclude], unclear [exclude]

2.4.2 Data Extraction

A Microsoft Excel® spreadsheet will be used to extract data by independent reviewers working in pairs. Prior to extraction, a calibration exercise will be carried out using a minimum of 10% of randomly chosen papers.

The following information will be extracted from research that qualify:

General Information

- Name of the first author
- Publication year
- Study duration
- Country and specific location of sample collection
- Study design (e.g., candidate gene association study, GWAS, QTL mapping, controlled challenge trial, functional knockout/overexpression study)
- Funding source

Population Data

- Chicken breed or population (commercial lines, indigenous/local breeds, inbred MHC-defined lines, crossbreds, or cell lines)
- Production system (backyard, semi-intensive, intensive) where applicable
- Number of farms sampled (where applicable)
- Number of birds sampled or experimental units
- Sampling method (random, systematic, convenience)
- Sample size

Exposure and Outcomes

- Target gene(s) and specific polymorphisms/markers examined (e.g., Mx G/A SNP, MHC B-F exon 2 SNPs, B-LBII loci G97A/T138A, LEI0258 microsatellite alleles, ROBO2 SNPs 1418G>A/2462T>C)
- Genotyping methods (PCR-SSCP, Sanger sequencing, genotyping arrays, etc.)
- NDV challenge or vaccination protocol (strain type, dose, route, time points)

- Newcastle disease outcomes measured: antibody titres (HI or ELISA), viral load/titre, viral shedding, clinical scores, mortality/survival, T cell proliferation/activation, body weight change post-infection
- Key findings: direction of associations, statistical significance, effect sizes (percentage of variance explained, odds ratios, genotypic means ± SD) where reported
- Risk factors associated with resistance or susceptibility genotypes (if reported)

2.5. Risk of Bias Assessment

The quality of each study will be assessed narratively across the following domains, informed by critical appraisal checklists (Munn *et al*., 2014):

- Sample size and statistical power
- Clarity and reproducibility of genotyping methods
- Standardisation of NDV phenotype measurement (challenge protocol, assay methods, time points)
- Correction for multiple testing (GWAS/QTL studies)
- Population characterisation (breed, relatedness, stratification) and correction for population structure

Each study will be rated as having low, moderate, or high risk of bias based on specific criteria. If needed, based on the study design, other quality assessment tools will be used. This assessment will be conducted by at least two independent reviewers.

2.6. Data Synthesis

The PRISMA 2020 guidelines will be followed during the review (Page *et al*., 2021). Given the substantial heterogeneity in study designs, chicken populations, genotyping methods, NDV challenge protocols, and outcome measures, formal statistical meta-analysis will not be performed. Results will be presented as a narrative synthesis, organised by candidate gene category (Mx gene, MHC polymorphisms, ROBO2, and other GWAS/QTL-identified loci).

Patterns across studies will be discussed qualitatively, including:

- Consistency of associations across populations and breeds
- Population specificity of resistance-conferring alleles or haplotypes
- Pleiotropic effects on production traits and susceptibility to other pathogens
- Gene-by-environment interactions (e.g., heat stress effects on genetic associations)
- Methodological quality considerations and strength of causal evidence

If sufficient data are available (four or more studies reporting on the same gene-outcome combination), pooled estimates may be produced. Subgroup analyses will take into account factors such as:

- Geographic region (Africa, Asia, North America, Europe)
- Chicken breed or population type (commercial, indigenous, inbred lines, crossbreds)
- Study design (candidate gene association, GWAS, QTL mapping, functional study)
- NDV strain type (lentogenic vs. velogenic) and challenge protocol
- Outcome measurement method (ELISA vs. HI; different time points)

For all analyses, statistical significance will be set at $p < 0.05$. If quantitative synthesis is not feasible due to high heterogeneity or insufficient data, findings will be reported descriptively with tabulation of study characteristics and key results.

2.7. Sensitivity Assessment

The I^2 statistic and Cochran's Q test will be used to evaluate heterogeneity where meta-analysis is feasible. Subgroup analysis and meta-regression will be used to investigate possible sources of heterogeneity if it is high ($I^2 > 50\%$, $p > 0.05$). To evaluate the robustness of the results, sensitivity analyses will be performed by eliminating studies with a high risk of bias.

2.8. Reporting Bias Across Studies

A funnel plot and Egger's regression test will be used to assess publication bias if the meta-analysis contains at least ten studies (Mavridis et al., 2014). A significant Egger's test result ($p < 0.05$) or asymmetry in the funnel plot will suggest possible publication bias. Given the relatively small number of studies anticipated for ROBO2 and novel GWAS loci, formal assessment of publication bias may be limited.

3. Conclusions

This systematic review aims to provide a comprehensive assessment of candidate gene polymorphisms associated with Newcastle disease outcomes in chickens. The findings will highlight the current state of genetic resistance markers, identify the predominant resistance-conferring alleles and haplotypes, and reveal population-specific effects across different chicken breeds and geographic regions.

The results will inform poultry breeders, geneticists, and veterinary services about the genetic basis of NDV resistance, helping to prioritise resources for marker-assisted selection and genomic selection programmes. The review will identify gaps in current research and monitoring efforts, guiding future studies on this important One Health issue at the interface of animal health, food security, and sustainable poultry production.

Additionally, the synthesis will evaluate the reproducibility of genetic associations across diverse populations, assess the strength of causal evidence (including functional validation through gene editing), and discuss the practical implications for breeding programme design in both commercial and smallholder poultry production systems, particularly in resource-limited settings where infrastructure-independent genetic improvement strategies are critically needed.

4. References

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Abbreviations

AOAV-1: Avian orthoavulavirus 1

APMV-1: Avian paramyxovirus 1

ELISA: Enzyme-Linked Immunosorbent Assay

GWAS: Genome-Wide Association Studies

HI: Haemagglutination Inhibition

MHC: Major Histocompatibility Complex

Mx: Myxovirus resistance gene

ND: Newcastle Disease

NDV: Newcastle Disease Virus

PCR-SSCP: Polymerase Chain Reaction – Single-Strand Conformation Polymorphism

QTL: Quantitative Trait Locus

ROBO2: Roundabout Guidance Receptor 2

SNP: Single Nucleotide Polymorphism