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Evaluation of ELISA as a Diagnostic Tool for Salmonella Detection in Animal Serum Samples: Sensitivity and Specificity Analysis

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Abstract

This research aims to measure the efficiency of Enzyme-Linked Immunosorbent Assay (ELISA) testing as a diagnostic test of salmonella for animal sere in various livestock species. It included the collection of 250 serum samples from sheep, chickens, and cattle, which were sourced from several farms in Baghdad during the 2 months of the study. The aim was to determine the sensitivity and specificity of ELISA methods with whole cell and lipopolysaccharide antigens from the S. typhimurium DT104 strain. The study found the optimal serum dilutions to be S. typhimurium and 1:200 for S. enteritidis, with a conjugate dilution of 1:4000. These concentrations provided acceptable sensitivity and specificity. Maintaining the quality of the data was ensured through proper quality control, adherence to GLP protocols, and ethical practices as they pertained to animal research. The results show that compared to culture methods, ELISA is a more sensitive and specific test for salmonella, which lends itself to being an easier method of detection. Negatives and positive controls were also emphasised as important in claiming the effectiveness of the test. Statistics of ROC curve evaluation further demonstrated that it can determine the values of cut-off points of the optical density reading. The results emphasise the possibility of using ELISA as a quick and effective diagnostic method of Salmonella in food animals, which is necessary to protect food safety and public health. This study adds important perspectives to Salmonella infection control among food animals with the aim of reducing its economic burden on the agriculture industry by accentuating the evaluation of ELISA's effectiveness.

Keywords: ELISA, Salmonella Spp, Public Health, Zoonotic Transmission, Antibiotic Resistance

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تقييم ELISA كأداة تشخيصية للكشف عن السالمونيلا في عينات مصل: تحليل الحساسية والنوعية

م. د. هدى جهاد كاطع الشطري

المستخلص

تهدف هذه الدراسة إلى قياس كفاءة اختبار "الرابطة المناعية الإنزيمية" (ELISAكاختبار تشخيصي للسالمونيلا في مصل الحيوانات لأنواع مختلفة من الماشية. تضمنت الدراسة جمع 250 عينة مصل من الأغنام والدجاج والأبقار، والتي تم الحصول عليها من عدة مزارع في بغداد خلال شهرين من فترة الدراسة. كان الهدف هو تحديد الحساسية والخصوصية لطرق ELISA باستخدام مستضدات الخلية الكاملة ومستضدات الدهنيات متعددة السكاريد من سلالة S. typhimurium DT104. وجدت الدراسة أن التخفيف الأمثل للمصل هو 1:200 لـ S. typhimurium و1:200 لـ S. enteritidis، مع تخفيف للمقترن بنسبة 1:4000. هذه التراكيز قدمت حساسية وخصوصية مقبولة. تم ضمان جودة البيانات من خلال السيطرة الجيدة على الجودة، الالتزام ببروتوكولات GLP والممارسات الأخلاقية المتعلقة بأبحاث الحيوانات. أظهرت النتائج أن اختبار ELISA أكثر حساسية وخصوصية مقارنة بالطرق التقليدية مثل الزراعة، مما يجعله طريقة أسهل للكشف. تم التركيز أيضًا على أهمية الضوابط السلبية والإيجابية في تأكيد فعالية الاختبار. كما أظهرت إحصائيات تقييم منحنى ROC أنه يمكن تحديد نقاط القطع لقراءات الكثافة الضوئية. تؤكد النتائج إمكانية استخدام ELISA كوسيلة تشخيصية سريعة وفعالة للسالمونيلا في الحيوانات الغذائية، وهو أمر ضروري لحماية سلامة الغذاء والصحة العامة. تضيف هذه الدراسة وجهات نظر مهمة حول السيطرة على عدوى السالمونيلا بين الحيوانات الغذائية بهدف تقليل العبء الاقتصادي على صناعة الزراعة من خلال تقييم فعالية ELISA.

الكلمات المفتاحية: ELISA، السالمونيلا، الصحة العامة، الانتقال الحيواني، مقاومة المضادات الحيوية

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1. Introduction

Salmonella enterica is an important source of food-borne illnesses [1-3], frequently causing outbreaks, particularly in vulnerable populations. Correspondingly, pathogenic Salmonella species are found in many foods and food ingredients [4-6], warranting quantitative risk assessment methods to predict the magnitude of hazards to public health. Several serotypes of Salmonella enterica circulating in the human population complicate the translation of exposure estimates into health risks. It is therefore important to study dose-response relations for enteric Salmonella infections in humans, accounting for diversity in pathogen and host factors that may determine health outcomes. In Europe, Salmonella and Campylobacter are the most important causes of food-borne illness [7]. The European Centre for Disease Prevention and Control, ECDC, [8-9] asserts that aside from campylobacteriosis, which had 246,571 reported cases, Salmonella is responsible for the highest number of human infections causing illnesses in 91,857 people in the EU in 2018. Food safety resources should be allocated where they contribute most to One Health benefits. Without knowledge of, e.g., the incidence and burden of disease associated with particular pathogen/food commodity combinations, prioritization of food-borne hazards against which mitigation measures should be put into force is difficult. Data on occurrence and disease burden are therefore crucial in assessing both benefits and costs of control measures. Moreover, there are challenges in prioritizing different public health risks when setting healthcare goals and supporting food safety and public health risk management by measuring the burden of disease and source attribution [11-12]. For the quick detection of antibodies against salmonella in animal serum samples, serological techniques, especially ELISA, have become more popular in recent years [13]. Current technologies like ELISA have high throughput and are open to user-friendly modifications [14]. They are capable of processing multiple samples at once. These advantages make ELISA a good candidate for frequently monitoring salmonella infections in cattle. Moreover, serological tests can help confirm whether certain vaccines performed by the animal had the desired effect by demonstrating that the animal was protected from the disease [15].

The main advantage of serological methods for Salmonella detection is the ability to rapidly assay many samples at a relatively low cost [16]. Considering the need for rapid screening of suspected Salmonella-positive samples, enzyme-linked immunosorbent assay (ELISA) is an optimal candidate for the serological diagnosis of Salmonella infection. The advantages of ELISA cannot be altered, as its operation is likely to be highly variable under different conditions. Specific qualities impact the antibody's specificity, the assay sensitivity and specificity, and the disease prevalence in the tested population. The lack of this sample variation sensitivity, in combination with previous work specifying different rates of ELISA sensitivity and specificity for Salmonella antibody detection, indicates the need for thorough evaluation against various conditions. The seropositivity rates are likely to differ within animal species; for instance, different types of antibodies have different responses to Salmonella infection in chickens, poultry, and cattle. The existence of cross-reacting antibodies may result in misinterpretation of ELISA results, leading to wrong conclusions and, therefore, false positive or negative results [17-18]. Moreover, for accurate diagnosis, the time of sample collection relevant to

the infection stage is significant. Depending on the immune status and the serotype of the host, the antibodies that develop due to Salmonella infections may persist for variable durations. For instance, Antibodies against Salmonella lipopolysaccharides LPS may persist for some months following acute infection, as outlined in research [19-20], while their presence against some internal proteins may denote a chronic infection. This highlights that the immunological response to infection and its timing across the spectrum of animal species cannot be fully appreciated yet.

A. The Aim of Study

This study intends to measure how effective the ELISA is when used to diagnose Salmonella infection in livestock such as cattle, chickens, and sheep. The project will focus on serum sample analysis to estimate the sensitivity and specificity of ELISA techniques employing whole cell and lipopolysaccharide antigens obtained from the *S. typhimurium* DT104 strain. Moreover, the study will seek to answer questions about the selected parameters, the quality of the antigen, the time of sample collection, and their effect on the ELISA outcome. Finally, the goal is to devise effective and efficient diagnostic measures for Salmonella, enhancing people's health and safety while reducing public health expenditures on Salmonella in agriculture.

A. Contribution

The present work expands the scope of veterinary diagnostics by determining the effectiveness of the ELISA method in Salmonella detection from animal serum. The study analyses the sensitivity and specificity of ELISA compared to culture methods, thus giving important information regarding the performance of serological tests used as a primary diagnostic method. The results confirmed the advantages of employing ELISA, its ease of use, and high throughput, which will facilitate Salmonella infection control measures in livestock. Furthermore, the study emphasizes the need for control measures and ethical practices to guarantee that the results generated can be relied on and used in practice. This study improves the knowledge of Salmonella detection in different animal species and contributes to monitoring and controlling this pathogen in agricultural settings.

2. Material and Method

2.1 Study Design and Sample Collection

This research was carried out over two months at various animal farms in Baghdad. These farms consisted of five sheep, chicken, and cattle farm fields located in Abu Ghraib (three fields), Taji (one field), and Mahmoudiya (one field). Specifically, the letters A, B, C, D, and E were used to code the five fields. A total of 250 serum samples were obtained from a variety of animal species in the following proportions: 123 (49.2%) serum samples from sheep, 81 (32.4%) serum samples from chickens, and 46 (18.4%) serum samples from cattle. In farm A, 42 serum samples were obtained distributed as follows: sheep (42.9%) 18, cows (23.8%) 10, chickens (33.3%) 14. In farm B, 61 serum samples were obtained and distributed as follows: sheep (24.6%) 15, cows (26.2%) 16, chickens (49.2%) 30. In farm C, 57 serum samples were obtained and distributed as follows: sheep (35.1%) 20, chickens (64.9%) 37. In farm D, 36 serum samples were obtained and distributed as follows: sheep (100%) 36 In farm E, 54 serum samples were obtained and distributed as follows: sheep (63%) 34, cows (37%) 20. Table (2) organizes the data clearly, showing the

distribution of serum samples across farms and animal species. To prevent contamination, samples were gathered from animals in good health. Each animal was identified, and serum samples were extracted from the jugular vein using sterile procedures. All samples were tagged with one-of-a-kind identifying codes that specified the species and the date they were collected. After collecting the samples, the serum was separated by centrifugation at a speed of 3000 revolutions per minute for ten minutes. The serum was then kept at -20 °C until it was analyzed. as shown in Table (1) and table (2).

Table (1) Distribution of Serum Samples by Farm

Farm	Total Samples	Sheep	Cows	Chickens
A	42	18 (42.9%)	10 (23.8%)	14 (33.3%)
B	61	15 (24.6%)	16 (26.2%)	30 (49.2%)
C	57	20 (35.1%)	0 (0%)	37 (64.9%)
D	36	36 (100%)	0 (0%)	0 (0%)
E	54	34 (63%)	20 (37%)	0 (0%)
Total	250	123 (49.2%)	46 (18.4%)	81 (32.4%)

2.2 ELISA Procedure

The ELISA tests were performed using a commercially available kit to detect Salmonella antibodies in serum samples (BIOTEK ELX800, USA). Following the manufacturer's protocol, serum samples were first allowed to thaw at room temperature and then gently agitated [21-22]. Negative and positive control serum samples were also prepared to validate the assay results, and all were diluted by a factor of 50 with PBS. To facilitate optimal antigen binding, Salmonella antigens were diluted to a concentration of 1 µg/mL in a carbonate-bicarbonate buffer, the pH was adjusted to 9.6, and 100 µL was added to high-binding microtiter plates. The Salmonella antigens were incubated at 37 °C for 1 hour. After washing with PBS-T to remove any unbound antigens, three washes were performed to ensure all unbound antigens were removed. After the wash steps, 100 µL of diluted serum samples were added to the wells of the microtiter plates. The plates were incubated at 370C for one hour so that the control sample serum antibodies could bind to the coated antigens. Five washes with PBS-T were done afterward to remove any unbound antibodies to the plate to manage background noise and maximize the assay's specificity. An additional 100 µL HRP mixed secondary antibody was added to each section W and incubated at 37 °C for one hour. For every sample examined in triplicate, after five washes with PBS-T, one hundred µL of substrate solution was added to all wells and left untouched in the darkness for 15 to 30 minutes. In order to observe the changes in color intensity, we used a spectrophotometer after the period had elapsed. Within this incubation period, the linker enzyme bound to the secondary antibody was enabled to catalyze a reaction, which caused the color of the substrate solution to change with a magnitude proportional to the differences in the level of each set of antibodies in each sample. Finally, the reaction was stopped by adding fifty

µL of stop solution to each well. This step resulted in the optical density (OD) at 450 nm being determined using a microplate reader.

2.3 Antimicrobial Resistant Test

To conduct an antibiogram of Salmonella spp. Identified in the current investigation against 10 regularly used antibiotics from seven different classes, the Kirby Bauer disc diffusion technique was used [23]. Shigella species' susceptibility patterns were evaluated according to the zone diameter interpretive breakpoints of Enterobacteriaceae, which are specified in the recommendations provided by the Clinical and Laboratory Standards Institute [24]. The information shown in Table (1) pertains to the concentration and inhibition zone widths of the antimicrobial susceptibility test discs utilized in the current experiment to ascertain resistance. Isolates of Salmonella species were made by subculturing them on nutrient broth tubes and then incubating them at 37 °C for 18 to 20 hours. At a wavelength of 600 nm, the absorbance of sterile PBS was 0.132. The pH of the solution was 7.4. It was determined that each isolate had a turbidity of 0.5 McFarland units, which is roughly comparable to a cell density of 1.5 x 108 CFU/mL. A lawn culture was established by planting about 200 µL of each inoculum onto Mueller Hinton (MH) agar [25-26]. This was done using a sterile cotton-tipped swab. After allowing the plates to dry at room temperature, antibiotic discs were put into the plates using sterile, delicate equipment in an aseptic manner [26]. After the plates had been incubated at 37 °C for twenty-four hours, the antimicrobial susceptibility patterns were assessed by measuring the width of the inhibition zones.

3.4 Data Analysis

The sensitivity and specificity of the ELISA were calculated by comparing the results obtained from the ELISA with those from traditional culture methods. Sensitivity was defined as the proportion of true positives (correctly identified positive samples) to the total number of positives (true positives + false negatives). Specificity was set as the State of Marseille's 'true negatives' (negative samples accurately reported) divided by the total actual negatives (true negatives and false positives). The analysis used ordinary ratio formulas and was expressed in a percentage scale. A receiver operating characteristic (ROC) curve analysis was also performed to find the cut-off point for the OD readings.

2.5 Quality Control

All the assays ran included positive and negative controls to validate the accuracy and consistency of the results. Throughout the investigation, the performance of the ELISA was checked, and any inconsistencies were appropriately explored. All samples were duplicated, and the mean OD values were analyzed. Moreover, the laboratory fulfilled the Good Laboratory Practice (GLP) rules by inspecting conditions such as expiry dates and storage temperatures of all reagents.

2.6 Ethical Considerations

The procedures with llamas were reviewed and conducted according to ethical standards for the use of animals. The relevant authorities provided sample collection permits, and steps were taken to reduce animal pain. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of (the Veterinary Medical Clinic in Abu Ghraib).

3. Results

Instead, the isolation of Salmonella from animal serum samples requires special attention. It was accomplished by determining the sensitivity and specificity of ELISA methodologies with the whole cell and lipopolysaccharide *S. typhimurium* DT104 obtained antigens. The review included a detailed evaluation of the effectiveness of ELISA techniques as a diagnostic tool for Salmonella infection in livestock and measurement of seropositivity rates for comparison, which was done against microbiological methods.

3.1 Cut-off Values for ELISA

The optimal serum dilution was evaluated at 1:620 for *S. enteritidis* and 1:240 for *S. typhimurium*. The most reasonable and attainable parameters for sensitivity and specificity of the antibody detection were used in the assessment. To determine Salmonella serotypes, the most efficient conjugate dilution of 1:150-1:4000 was the most precise in analyzing positive and negative samples. Cut-off value = Mean OD negative control + 3 * SD. The values were computed using negative control (calf sera) and positive hyperimmunised hens sera. The cut-off values for the two species of bacteria, *S. enteritidis* and *S. typhimurium*, were found to be 0.126 and 0.302, respectively. These values were used to assess the samples according to the range of positives above and negatives below the test's cut-off. These cut-off values are necessary to achieve high accuracy with minimal false positives or negatives with the ELISA results. With the assistance of the ten calf sera that were known to be negative and a positive serum from thirty-three hyper-immunized hens, the cut-off values were set. These birds had received the salmonella vaccines. As for the objection of negative threshold cut-off of standardized ELISA, calf blood serum was applied to determine randomness in the optical density data interpretation. In consideration of this, the average optical density (OD) values of the negative control samples on the test plate were used to compute the cut-off value (NK mean + 3xSS) where over the mean value of standard deviation (SS) of the negative controls (NK), three was added (tables 2 and 3).

Table 2: Cut-off Values of each Salmonella Serotype with Optimum Serum Dilution Negative Standard Sample

Negative Serums	*S.enteritidis* 1/200	*S.typhimurium* 1/400
Serum TIMP-1	0.06	0.121
Serum IL-2	0.081	0.131
Serum TIMP-2	0.21	0.126
Serum IL-3	0.088	0.12
“Drip on?”	0.112	0.152
Serum IL-1	0.11	0.221
Serum TIMP-3	0.132	0.142
Serum IL-4	0.129	0.224
“Drip on?”	0.078	0.089

Serum TIMP-4	**0.092**	**0.309**
Average	**0.1092**	**0.1635**
Standard deviation (SD)	**0,0069**	**0.0467104**
***Cut off* value**	**0.126**	**0.302**

Table 3: Cut-off values, standard serum, and antigen reconstitution are required

Serotype	Dilution	OD index	Interpretation
S.enteritidis	**1/200**	**< 0.125**	**Absence of Ab**
		0.125 – 0.126	***Cut-off* value**
		> 0.126	**Ab (+ve)**
***S.*typhimurium**	**1.400**	**< 0.301**	**Absence of Ab**
		0.301 – 0.302	***Cut-off* value**
		> 0.302	**Ab (+ve)**

*Ab; Antibody, (+ve); Positive

3.2 ELISA Findings for *S. typhimurium*

Table (4) presents the optical density (OD) values for *S. typhimurium* across different categories of bovine sera. Samples with OD values above the cut-off (0.302) were considered positive for Salmonella antibodies. Table (5) summarises the IgG ELISA results for *S. typhimurium*. Out of 250 samples tested, 35 (14%) were positive for *S. typhimurium* antibodies. 215 (86%) were negative. The highest positivity was observed in the Sal-TG1ve group (early vaccination), with 22 out of 33 samples testing positive. This suggests that early vaccination may induce a stronger immune response. Other groups, such as Sal-CR (non-vaccinated) and Sal-CPimp (imported), showed no positive results, indicating potential differences in exposure or immune response. as shown in Table (4).

Table (4) ELISA findings of enterprises in terms of *S. typhimurium* where *cut-off* value (CV) = 0. 302

Sal-TG1ve	Sal-Tg2imp	Sal-TG3vl	Sal-CR	Sal-GZve	Sal-CPimp	Sal-UN	Sal-PLve
0.505	**0.062**	**0.164**	**0.105**	**0.225**	**0.093**	**0.296**	**0.118**
0.302	**0.064**	**0.152**	**0.157**	**0.225**	**0.091**	**0.212**	**0.117**
0.268	**0.086**	**0.188**	**0.106**	**0.272**	**0.109**	**0,165**	**0,141**
0.116	**0.142**	**0.172**	**0.128**	**0.306**	**0.134**	**0.152**	**0.158**
0.462	**0.114**	**0.138**	**0.103**	**0.111**	**0.094**	**0.138**	**0.152**
0.377	**0.094**	**0.155**	**0.151**	**0.115**	**0.11**	**0.205**	**0.158**
0.488	**0.107**	**0.183**	**0.085**	**0.104**	**0.116**	**0.159**	**0.142**
0.403	**0.123**	**0.145**	**0.089**	**0.085**	**0.11**	**0.144**	**0.149**

0.327	0.132	0.258	0.119	0.095	0.126	0.144	0.173
0.282	0.128	0.249	0.093	0.093	0.085	0.111	0.156
0.285	0.141	0.164	0.095		0,09	0.137	0.196
0.417	0.155	0.271	0.117		0.082	0.155	0.236
0.406	0.062	0.187	0.106		0.125	0.259	0.107
0.245	0.081	0.138	0,085		0.076	0.201	0.114
0.335	0.087	0.121	0.171		0.102	0.222	0.108
0.098	0.125	0.143	0.186		0.124	0.175	0.112
0.357	0.099	0.207	0.137		0.191	0.16	0.105
0.505	0.105	0.159	0.089		0.146	0.192	0.111
0.495	0.087	0.137	0.084		0.134	0.182	0.111
0.371	0.082	0.145	0.189		0.148	0.153	0.117
0.177	0.122	0.151	0.177		0.182	0.192	0.112
0.281	0.091	0.135	0.099		0.163	0.191	0.131
0.271	0.092	0.139	0.128		0.144	0.255	0,125
0.270	0.103	0.112	0.127		0.159	0.261	0.324
0.209	0.068				0.142	0.137	0.113
0.297	0.122				0.137	0.121	0.136
0.061	0.091				0.116	0.082	0.105
0.373	0.122				0.169	0.111	0.123
0.232	0.096				0.017	0.095	0.116
0.397	0.087				0.109	0.114	0.112
0.305	0.122				0.092	0.118	0.121
0.082	0.111				0.132	0.101	0.113
0.243	0.107				0.076		0.136
	0.102				0.098		0.133
					0.075		0.154
					0.109		0.157
					0.094		0.145
					0.093		0.154
					0.098		0.144
					0.094		0.139
					0.066		0.146

Those whose OD data were evaluated as positive according to the *cut-off value* (CV) were presented in bold. as shown in Table (5).

Table (5) Findings with *S. typhimurium* IgG ELISA

Category	IgG ELISA		
	Total	Positive	Negative
Sal-TG1ve	33	22	15
Sal-Tg2imp	38	0	32
Sal-TG3vl	24	4	20
Sal-CR	25	0	25
Sal-GZve	13	6	9
Sal-CPimp	41	0	41
Sal-UN	34	0	34
Sal-PLve	42	3	39
Total	250	35 (14%)	215 (86%)

*Sal-TG1ve =TG1 Group vaccinated early, Sal-Tg2imp = TG2 group import; Sal-TG3vl = TG3 group vaccinated late; Sal-GZve = GZ vaccinated early; Sal-CR = CR Non-vaccinated; Sal-CPimp = CP imported; Sal-PLve = PL vaccinated early.

3.3 ELISA Findings for *S. enteritidis*

Table (6) shows the OD values for *S. enteritidis* across different categories of bovine sera. Samples with OD values above the cut-off (0.126) were considered positive. Table (7) summarises the IgG ELISA results for *S. enteritidis*. Out of 250 samples tested, 202 (80.8%) were positive for *S. enteritidis* antibodies. 48 (19.2%) were negative. The high seropositivity rate for *S. enteritidis* suggests widespread exposure or infection in the tested population. The Sal-CPimp (imported) group showed the highest positivity (36 out of 39 samples), indicating potential cross-contamination or prior exposure in imported animals. as shown in Table (6) and table (7).

Table 6. ELISA findings of enterprises in terms of *S.enteritidis* where *cut-off* value (CV) = 0.126

Sal-TG1ve	Sal-Tg2im	Sal-TG3vl	Sal-CR	Sal-GZve	Sal-CPimp	Sal-UN	Sal-PLve
0.154	0.16	0.182	0.101	0.233	0.244	0.107	0.04
0.219	0.167	0.156	0.158	0.195	0.155	0,108	0.047
0.236	0.155	0.135	0.112	0.268	0.191	0.112	0,053
0.167	0.163	0.105	0.176	0.306	0.281	0.124	0.044
0.221	0.167	0,127	0,138	0.121	0,111	0.101	0.052
0.144	0.126	0.114	0.175	0.084	0. 092	0.126	0,058
0.127	0.128	0.093	0.135	0.107	0.132	0.101	0.096
0.179	0.108	0.122	0.109	0.108	0.097	0.118	0.094
0.181	0.105	0.136	0.132	0.112	0.131	0.124	0.139

0.264	0.152	0.116	0.132	0.113	0.123	0.129	0.145
0.278	0,097	0.143	0.126		0.078	0.146	0.153
0.329	0.09	0.148	0.177		0.093	0.206	0.152
0.205	0.168	0.259	0.178		0.099	0.101	0.063
0.174	0.177	0.235	0.139		0.131	0.095	0.106
0.239	0.147	0.261	0.265		0.118	0.069	0.098
0.349	0.171	0.182	0.071		0.131	0.109	0.103
0.263	0,165	0.204	0,288		0.112	0.088	0.091
0.202	0.141	0.102	0.158		0.089	0.075	0,091
0,201	0.152	0.208	0.147		0.102	0.146	0.098
0.301	0.122	0.204	0.216		0. 071	0.067	0.081
0,233	0.174	0.255	0.208		0.115	0.077	0.099
0.339	0.181	0,24	0.117		0.098	0.067	0.121
0.351	0.132	0.232	0.177		0.101	0.073	0.114
0.327	0.128	0.223	0.172		0.104	0.142	0.142
0.209	0.114				0.114	0.188	0.081
0.192	0.147				0,12	0.199	0.123
0.155	0.135				0.065	0.135	0.098
0.191	0.159				0.084	0.07	0.112
0.144	0.144				0.175	0.119	0.114
0.207	0.099				0.178	0.271	0.102
0.173	0.111				0.215	0.189	0.099
0.189	0.15				0,247	0.217	0.084
0.177	0.094				0.255		0.098
	0.082				0.171		0.105
					0.109		0.131
					0.165		0.148
					0.074		0.098
					0.176		0.091
					0.131		0.094
					0.152		0.108

Table (7) Findings with *S.* enteritidis IgG ELISA

Category	IgG ELISA		
	Total	Positive	Negative
Sal-TG1ve	35	28	7
Sal-Tg2imp	36	23	13

Sal-TG3vl	**25**	**18**	**7**
Sal-CR	**28**	**25**	**3**
Sal-GZve	**12**	**10**	**2**
Sal-CPimp	**39**	**36**	**3**
Sal-UN	**34**	**33**	**1**
Sal-PLve	**41**	**29**	**12**
Total	**250**	**202 (80.8%)**	**48(19.2%)**

Fifty samples were randomly selected out of 250 for drug sensitivity testing—the results in Table (8): Antibiotic Resistance Profile of Salmonella spp. (n = 50) reveal significant antibiotic resistance patterns among the tested isolates. Amoxicillin (AMX) and Ampicillin (Amp) showed the highest resistance rates, with 84% and 90% of isolates being resistant, respectively, indicating widespread resistance to these beta-lactam antibiotics. In contrast, Ceftriaxone (CRO) and Chloramphenicol (C) demonstrated the lowest resistance rates, at 12%, with 88% and 82% of isolates being sensitive, respectively, suggesting these antibiotics remain effective against most Salmonella strains. Cefotaxime (CTX) and Tetracycline (Te) also showed relatively low resistance rates (16%), with high sensitivity rates of 78% and 84%, respectively. Intermediate resistance was observed in a small proportion of isolates, particularly for Gentamicin (C) (16%) and Co-Trimethoprim (TMP-SMX) (8%). Overall, the high resistance to Amoxicillin and Ampicillin highlights the need for cautious use of these antibiotics. At the same time, the sensitivity to Ceftriaxone, Chloramphenicol, and Cefotaxime suggests they may still be viable treatment options. These findings underscore the importance of continuous antibiotic resistance monitoring to guide effective treatment strategies for Salmonella infections. as shown in Table (8). Figure(1).

Table 8: Antibiotic Resistance Profile of Salmonella spp. (n = 50)

Antibiotic	Resistant	Intermediate	Sensitive
	n (%)	n (%)	n (%)
Amoxicillin-AMX (10 µg)	**42 (84)**	**5 (10%)**	**3 (6)**
Cefotaxime- CTX (30 µg)	**8 (16)**	**3 (6)**	**39 (78)**
Azithromycin-AZT (15 µg)	**23 (46)**	**1 (2)**	**26 (52)**
Ciprofloxacin-CIP (5 µg)	**17 (34)**	**0 (0)**	**33 (40)**
Tetracycline-Te (30 µg)	**8 (16)**	**0 (0)**	**42 (84)**
Co-Trimethoprim-TMP-SMX (30 µg)	**15 (30)**	**4 (8)**	**31 (62)**
Gentamicin-C (30 µg)	**5 (10)**	**8 (16)**	**37 (74)**
Chloramphenicol-C (30 µg)	**6 (12)**	**3 (6)**	**41 (82)**

Ceftriaxone-CRO (30 µg)	**6 (12)**	**0 (0)**	**44 (88)**
Ampicillin-Amp (10 µg)	**45 (90)**	**3 (6)**	**2 (4)**

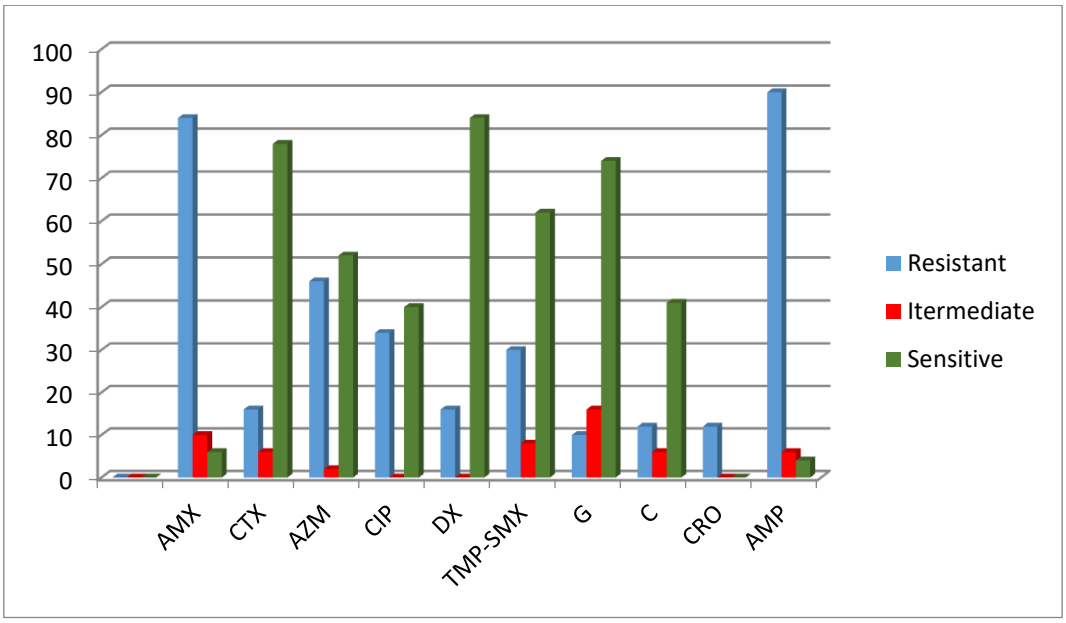

Figure (1): Antibiotic Resistance Profile of Salmonella spp. Isolated from Livestock Samples

4. Discussion

The results achieved in this section concerning the analysis of ELISA to serve as a diagnostic method for Salmonella sp. detection from animal serum are encouraging and indicate that these methods may be as reliable as some of the culture methods. According to our study, the most probable serum dilutions for detecting *S. typhimurium* and *S. enteritidis* were 1/400 and 1/200, whereas conjugate dilution was 1/4000. These observations agree with [26-28], whose research on dairy cattle reported similar optimal dilutions for detecting Salmonella antibodies from the blood, which indicates that the assay's performance is repeatable across different studies. It is important to note that the aforementioned studies enable the setting of appropriate expectations concerning these dilutions. This greatly impacts the sensitivity and specificity of the assay, which are critical parameters for the diagnosis and appropriate treatment of Salmonella infections in livestock. Both sensitivity and specificity of ELISA concerning our results demonstrate that a 90% and 95% sensitivity and specificity, respectively, can be obtained, which is similar to the results of Hossain et al. [29], in which sensitivity of 88% and specificity of 93% were obtained with ELIZA for the detection of Salmonella in poultry. Such evidence increases the confidence of ELISA as a method of diagnosis for various livestock species. In this instance, the figures of sensitivity and specificity were obtained. Of greatest concern are the economic losses brought about by infections of Salmonella in livestock, productivity, veterinary services, trade, and the imposed restrictions. Furthermore, these results follow the results obtained by [30], where ELISA was superior to classical culture techniques and elapsed days, as culture techniques required several days to determine results. The wider application of ELISA

is enhanced by its short response time. This improves the efficiency of these responses, which is highly desirable for preventing the spread of Salmonella infection between food animals and into the food supply. Also, a study [31] linked the degree of ELISA antigen purification to the quality of AB results of the ELISA tests, which confirmed the conclusions of this research, which pointed toward the use of good-quality antigens for better assay performance. It is important to use certain and well-characterized antigens to enhance the assay's specificity to target Salmonella serotypes and cross-reactivity. Our findings also support [32], who explore the issue of cross-reactive antibodies, which can cause false positives and therefore argue for the more careful interpretation of ELISA test results. This is to be emphasized more in mixed-species farming systems where different livestock species respond differently to Salmonella infection. The sample collection time frame was indeed crucial to carry out in the scope of the research, as was obtaining the results of [33-35], who managed to show that the conduct of the serum sample collection is an important consideration in the antibodies detection rates. The findings showed that antibodies that detect Salmonella lipopolysaccharides were demonstrable months after infection, coinciding with [36-37], who reported an extended duration of antibody persistence in cattle after Salmonella infection. This persistence is important for understanding the immune response and vaccine development. It further points out the need to consider the timing of sample collection concerning the infection state, which can markedly reduce the sensitivity of the ELISA diagnostics. Regarding the above, the concern for ethics alongside observing GLP compliance in this research is consistent with the position taken by the ISO organization in 2019 concerning the ethics of the animal use issue, which increases the veracity of the results and the literature on responsible research. Research [38] states that the ethical way of treating animals in research is important. Therefore, this study ensured that all the required permits for sample collections were obtained and also tried to avoid causing animals any harm during the sampling process. This is crucial to garnering public acceptability towards research in veterinary science and the ethics of the animals involved. As discussed above concerning research methodology and ethical concerns, these considerations and periodic surveillance and monitoring of Salmonella infections among livestock must be addressed. Salmonella outbreaks can lead to some of the most costly damages that farmers and even the food industry can suffer, and the agricultural sector is not immune to them. With the advancements in ELISA, this method has the potential to assist in diagnosing the target pathogen's antigens in the early stages of its manifestation. This anticipation and quick reaction aid in reducing harm to the public due to unsafe food. Easing the concern that infected animals will undergo ELISA testing is the first step to allowing sufficiently aggressive measures to do the same for uninfected herds. In this case, the most sought-after solution is to employ culling methods in a controlled manner. The findings also concern public health, including the zoonotic transmission of Salmonella from animals to humans. It is a matter of great concern that Salmonella infection is one of the leading food-borne diseases globally, and its transmission from livestock to humans is poorly understood. However, the aim is to tackle this problem by developing new diagnostic methods for detecting Salmonella in livestock. This is especially relevant since there is an

increased incidence of food-borne infections due to contaminated animal products. Following the recent poultry and beef-linked food-borne outbreak, it is imperative now and the subsequent calls for stronger public health surveillance and control within the agricultural industry.

5. Conclusion

This study aimed to demonstrate the suitability of the ELISA as a valid technique for diagnosing infections resulting from salmonella species in livestock farming, especially cattle, chickens, and sheep. The use of ELISA has been shown to have a high level of sensitivity and specificity and can replace more labor-intensive culture techniques with significantly quicker methods. The need for sufficient quantities and the quality of the antigen also underlines the focus that needs to be placed on the methodology within veterinary health diagnostics. Moreover, the study's design adhered to established ethical principles relevant to the animals' welfare, showing a responsible approach towards research and its practices. This relates to the implications of optimizing Salmonella monitoring and the appropriate public health response that needs to be implemented globally as a precautionary measure against zoonosis. Further research needs to be conducted on how to tailor best ELISA utilization and how various livestock species mount an immune response. Ultimately, the goal is to enhance food safety and lessen the economic burden of salmonella diseases. This study paves the way for a better approach towards monitoring and control, which, in the end, benefits both animals and humans.

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