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Detection of Human-Transmissible Animal Viruses in Meat Markets: A Survey Using PCR Techniques

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Abstract

This study aimed to assess the prevalence of animal viruses transmissible to humans in meat products traded in Iraqi markets, using polymerase chain reaction (PCR) techniques. Sixty equal samples were collected from three types of meat: poultry, red meat, and fish (20 samples for each type). The study focused on detecting hepatitis E viruses, adenovirus, and rotavirus, as these are among the viruses most associated with foodborne illnesses. The results showed a high percentage of contaminated samples, with 71.7% of samples testing positive for viruses, particularly HEV, AdV and RV reflecting the seriousness of markets with poor sanitary conditions. Analysis revealed differences in contamination rates depending on the type of meat, with poultry and red meat being more susceptible to contamination. PCR was also proven effective as a sensitive and accurate method for the early detection of foodborne viruses. The study concluded that markets that lack regular health supervision represent a fertile environment for the transmission of viruses from animals to humans, posing a threat to public health. The findings were supported by comparison with similar studies in Brazil and Laos, which emphasized the seriousness of open markets as a major source of viral epidemics. The study recommends strict market controls, the implementation of periodic PCR testing programs, and increased health awareness among consumers and food chain workers.

Keywords: Meat, PCR, Animal Viruses, Wet Markets

Introduction

In recent years, there has been an increase in the incidence of foodborne diseases worldwide, with viruses now being recognized as a major cause of these illnesses. The most common viruses implicated in foodborne disease are enteric viruses, which are found in the human gastrointestinal tract, excreted in human feces and transmitted by the fecal-oral route. Many different viruses are found in the gastrointestinal tract but not all are recognized as foodborne pathogens. The diseases caused by enteric viruses fall into three main types: gastroenteritis, enteric ally transmitted hepatitis, and illnesses that can affect other parts of the body such as the eye, the respiratory system and the central nervous system leading to conjunctivitis, poliomyelitis, meningitis and encephalitis. Viral pathogens excreted in human feces include noroviruses, sap viruses, enteroviruses, adenoviruses, hepatitis A virus (HAV), hepatitis E virus (HEV), rotaviruses, and astroviruses. Most of these viruses have been associated with foodborne disease outbreaks. Noroviruses and HAV are commonly identified as foodborne causes of gastroenteritis and acute hepatitis, respectively (Greening, 2016).

Beef, mutton, chevon, venison, fish and poultry meat act as a main sources of animal protein, Foodborne viruses are generally very infectious and their spreading are rapidly from one individual to the next, although several exceptions are exist as hepatitis E virus. The most of foodborne viruses outbreaks were linked with the infected food handlers, as hepatitis A virus are mainly transmitted between humans (Feagins et al., 2008). In contrast hepatitis E virus has been identified as an important disease. Beef, mutton, chevon, venison, fish and poultry meat can potentially be contaminated throughout the whole food product chain and sources of contaminations can include equipment, other contaminated food and Beef, mutton, chevon, venison, fish and poultry meat or meat products, originating from infected animals and water (Sobhy & Shaltout, 2020).

Beef, mutton, chevon, venison, fish and poultry meat act as a main sources of animal protein, and shellfish are the major food categories involved in foodborne of viral gastroenteritis origin (Kingsley & Chen, 2009). However, risky Beef, mutton, chevon, venison, fish and poultry meat are considered particularly those that are intended for direct consumption or that are not properly heat treated before consumption (Holzmann et al., 2009).

Other human- and animal-derived viruses, which have the potential to be transmitted by food, do not always infect the gastrointestinal tract. For example, HAV and HEV infect the liver; enterovirus, poliovirus, Nipah virus and tick-borne encephalitis virus infect the nervous system; and the SARS (severe acute respiratory syndrome) and the MERS (Middle East respiratory syndrome) coronaviruses and avian influenza virus H5N1 infect the respiratory system.

Five of the enteric viruses, namely norovirus, HAV, sapovirus, rotavirus and astrovirus, are included in the 31 major foodborne pathogens identified by the Centers for Disease Control and Prevention (CDC) (Scallan et al., 2011). Of the estimated 37 million foodborne illnesses that occur annually in the United States, viruses cause 59% of illnesses with noroviruses being the greatest contributor, causing an estimated 5.5 million cases per year. The European Food Safety Authority (EFSA) identified norovirus (NoV) and HAV as the major foodborne viruses of public health significance. The HEV was also included because of its high prevalence in pigs and the potential for zoonotic transmissionSeveral virus-commodity combinations were identified as high priority for implementation of prevention and control measures: a) NoV and HAV in bivalve molluscan shellfish; b) NoV and HAV in fresh produce; c) NoV and HAV in prepared (ready-to-eat) foods; d) Rotaviruses in water for food preparation; e) Emerging viruses in selected commodities

With the exception of shellfish, which are most often contaminated by sewage infiltration of harvesting waters, food handlers are the most significant source of viral foodborne illnesses. Foods most often implicated include salads, fresh produce and bakery or delicatessen items that are prepared or handled raw or after the foods have been cooked. The EFSA now recommends a focus on measures such as encouraging good hand hygiene and sanitation to prevent viral contamination of foods rather than virus removal or inactivation after foods have become contaminated (EFSA, 2011).

Research Problem

The increasing prevalence of viral zoonoses transmitted through animal meat poses a serious public health concern, particularly in traditional wet markets where sanitary regulations are often inadequate or poorly enforced. Despite the growing evidence of zoonotic viruses such as hepatitis E virus (HEV), adenovirus (AdV), and rotavirus (RV) being present in meat products, routine surveillance using sensitive molecular methods remains limited in many countries. This

gap in detection capacity allows potentially harmful viruses to enter the human food chain unnoticed, increasing the risk of outbreaks. The study seeks to address this problem by applying PCR-based detection techniques to assess the presence of human-transmissible animal viruses in commonly consumed meat products across selected meat markets.

Research Objectives

1. To detect the presence of human-transmissible animal viruses in meat samples using PCR techniques.
2. To identify which types of meat (e.g., pork, poultry, beef, fish) are more likely to contain viral contaminants.
3. To evaluate the correlation between market sanitary conditions and the prevalence of zoonotic viruses.
4. To recommend strategies for improving viral surveillance and food safety in meat markets.

Research Questions

1. Can animal viruses that can be transmitted to humans be detected in different meat samples?
2. Do PCR test results vary depending on the type of meat?
3. Do PCR results vary between different meat markets?

Study hypotheses

Hypothesis 1: Animal viruses transmissible to humans can be detected in various meat samples.

Hypothesis 2: PCR test results may vary depending on the type of meat.

Hypothesis 3: PCR results may vary between different meat markets.

Study community and sample

In the Iraqi cities of Baghdad and Basra, samples were gathered from neighborhood markets. The markets were chosen with care to represent a range of countries with respect of size and product type. There were 60 samples of various meats in the sample, evenly split between 20 samples of white meat (poultry), 20 samples of red meat, and 20 samples of fish.

The samples were gathered from the two cities' wholesale and commercial markets on December 2024 and February 2025. A type of animal viruses that can infect humans was found using the PCR (polymerase chain reaction) techniques.

PCR Primers

Specific primers were used for each virus we aimed to detect. The primers were selected based on their specificity and efficiency in amplifying viral genome. The viruses targeted in this study included:

HEV virus

Forward Primer: 5’-ATGAGAGTCTTCTTTGTC-3’

Reverse Primer: 5’-CCTGAGGTCATGTGAG-3’

Rotavirus:

Forward Primer: 5'-GGCTTTAAAAGAGAGAATTTCCGTCTGG-3'

Reverse Primer: 5'-GGTCACATCATACAATTCTAATCTAAG-3'

Adeno virus

Forward Primer: 5'-GCCACGGTGGGGTTTCTAAACTT-3'

Reverse Primer: 5'-GCCCCAGTGGTCTTACATGCACATC-3'

PCR Reaction Setup

The PCR reactions were carried out using a standard 25 µL reaction volume.

PCR Buffer: 1X

dNTPs: 0.2 mM each

MgCl2: 2.5 mM

Taq Polymerase: 1 U

Template DNA: 1 µL (extracted from meat samples)

Primers: 10 pmol of each forward and reverse primer

Thermal Cycling Conditions:

The thermal cycling protocol for all viruses was as follows: a) Initial denaturation: 95°C for 5 minutes; b) Denaturation: 95°C for 30 seconds; c) Annealing: 55°C for 30 seconds (optimized for each primer pair); d) Extension: 72°C for 1 minute; e) Final extension: 72°C for 10 minutes

Analysis

PCR products were analyzed using **gel electrophoresis** on a 1.5% agarose gel stained with **ethidium bromide**, and the bands were visualized under UV light to confirm the presence of the target viruses.

The areas and collection techniques were chosen using scientific criteria, with a focus on avoiding cross-contamination while collecting. To guarantee data accuracy and relate the findings to market conditions, specific information was documented for every sample, including the type of meat, location of collection, and the duration of sale.

Significance of the Study

This study provides critical insights into the risks associated with consuming meat from informal markets, where regulatory oversight is limited. It highlights the potential of PCR-based diagnostics in early detection of zoonotic viruses, contributing to public health protection, especially in low- and middle-income regions. The findings support global One Health initiatives by linking animal-source food safety with human disease prevention and offering evidence-based recommendations for improving meat inspection protocols and food hygiene practices.

Background on Animal Viruses

Animal viruses are crucial emergent pathogens that can jump species barriers, leading to global health and economic challenges. Recent zoonotic pandemics, while manageable with specific vaccines, have posed significant threats to vulnerable populations. Understanding these new viruses is essential for assessing risks to food safety and public health, which allows for the development of prevention and control measures. However, many needs remain unmet (Mendonça Soares et al., 2022). Traditional detection methods primarily identify known

viruses, which limits the detection of new ones. Hence, broad-range detection and follow-up studies for unknown viruses are urgently needed, aided by advances in synthetic biology, sequencing, and high-throughput technologies that enable metagenomics in virology and environmental studies (Kohl et al., 2015).

Numerous novel viruses have been detected, including broad-spectrum types, yet only a few have been used to screen huTAVs. Among the traded amphian and mammalian food animals, swine, minks, and notably yuan-yang and raised ducks were prioritized for studies on zoonosis

Types of Animal Viruses

The study screened adenoviruses, rotaviruses, and hepatitis E viruses using PCR techniques. DNA extraction was performed on 250 mg of fresh or frozen meat samples for PCR assays. The PCR reaction volume was 25 µl, with 20 µl of product analyzed via agarose gel electrophoresis and visualized with gel red. To detect adenovirus in meat cuts, primers targeting the AdV hexon gene were used, For HEV virus detection, specific primers for the ORF2 region were utilized and for rotavirus detection, specific primers for the VP6 region were utilized. Each sample included a negative control (H2O) and a positive control (Mendonça Soares et al., 2022).

Human-Transmissible Viruses

The highly pathogenic avian influenza virus (HPAIV) subtype H7N1 was linked to a turkey slaughterhouse outbreak in Italy in 1999. This virus transmitted to ferrets via meat, demonstrating its resilience during processing and storage. Analyses found that detecting AIV in contaminated food was more effective using alternative organ systems than the Dewu method, and food processing enzymes had no impact on viral infectivity (1990- Murcia Rodriguez, 2018). HIV-1 is believed to originate from the simian immunodeficiency virus (SIV) in primates, with SIVcpz being the precursor of the dominant HIV-1 group M. Studies suggest SIV can persist in pork, raising concerns about animal-to-human transmission, including potential SIVcpz transfer via pork products (Mendonça Soares et al., 2022).

Importance of Meat Markets

About 90% of global trade in animal products occurs in wet markets, particularly in East and South East Asia. These markets provide fresh seafood, meat, and poultry to urban populations, preserving cultural traditions and local trade. However, they are linked to outbreaks of zoonotic diseases (Senvanpan et al., 2023).

Role in Public Health

In analysis of meat products in several urban markets, the majority of viral agents detected were food-borne viruses HAdV and HEV, and these viruses also associated with meat consumption in diverse regions. Generally, there are several transmissions associated with foods leading to acute infectious diseases in humans. However, most studies also focused on the detections of these viruses remain in environmental or wastewater samples, and the virus detections in foods are lacking, especially in meat products (Mendonça Soares et al., 2022). This study highlights the importance of the potential pathogens, providing a comparative study on the application of PCR techniques, and emphasizing the necessity of monitoring food products in relation to public health.

Public markets are critical for food distribution in many developing countries. Unfortunately, some of these environments can also serve as potential sources of human viral pathogens. Modern reactor-based technologies for long-term continuous fluoroquinolone monitoring

were developed. About 89% of fecal relevant species were identified in an Italian river in this work and 69% in the river Seine.

Economic Impact

Although there is limited awareness of the One Health concept in LMICs, especially at the consumer level, considerable groundwork is being accomplished. The informal nature of meat markets makes them challenging places to work for researchers and health agencies. Although avian influenza is traditionally one of the most high-profile and studied pathogens in such settings, meat markets also present significant but less appreciated risks of zoonotic spillover. Owing to the multiplicity of factors involved in the viral contamination of food, it is essential to choose generic primers focusing on the detection of the viral family or multiple genotypes to detect genomes from different sources (Mendonça Soares et al., 2022). Food contamination can occur in several stages and is related to how they are handled during preparation and the environmental conditions.

The mode of actions for viruses in the genesis of diseases varies by virus species, with some causing damage to gastrointestinal epithelium and others causing an immunological hypersensitivity involved in extra-intestinal diseases. Meat is consumed across the world, but meats from different animal species vary between cultures, and the meat animal species consumed in Brazil differ from those consumed in other countries (Senvanpan et al., 2023). Despite the findings presented here, this project could be substantially improved. A classic detection strategy based on the amplification of a target of known sequence by PCR amplification and detection by hybridization was chosen.

Most One Health initiatives are targeting human health, thus eschewing the 'other' sacrosanct interconnections of animal health and environmental health. Limiting human population growth should be recognized as a necessary albeit challenging intervention for mitigation of biodiversity loss and zoonotic diseases. Limiting populations of domestic animals, however, has a notable contagion for livelihoods, foreshadowing an equally difficult challenge for policy and governance. Innovative and tractable strategies must be developed which incentivize and enable lower fertility rates in both spheres, and which enhance trade-offs that shift incentives for modeling, monitoring, and managing spillover risks.

Interventions with the potential to reduce transmission risk to humans and provide a range of environmental and social benefits include restoration of habitats across the landscape and creation of biodiversity banks which would offset resource extraction with improvements to conservation and climate change parameters. Specifically targeting conservation reserves and forests for planting of lost vegetation would enhance the resilience of the ecosystem and assist in maintaining wildlife populations that support diverse cultures and provide a valuable source of ecotourism.

PCR Techniques Overview

Polymerase chain reaction (PCR) techniques emerged in the late 1980s and soon hybridization probes (i.e., oligonucleotide probes and fluorogenically labeled probes) used for the detection of the amplification represented the beginning of every PCR system also termed as PCR. The PCR techniques have been used extensively in clinical diagnostics and basic research. This paper provides a review on the analysis and application of PCR based on the comparison of different probes, such as oligonucleotide probes, fluorescent DNA – nucleotide probes,

molecular beacons, and the magnitude of fluorescence from different detection materials (Rodriguez, 2005).

Principles of PCR

Polymerase chain reaction (PCR) is a technique used to amplify specific sequences of DNA. It is one of the most important tools in molecular biology, and is used in many applications, including cloning, gene isolation, sequencing, and diagnosis of infectious diseases. PCR involves thermal cycling, where the DNA is first denatured, then annealed, and finally extended. There are three basic steps in a PCR: denaturation, annealing, and extension.

PCR uses the ability of a DNA polymerase to synthesize a new strand of DNA complementary to the template strand. To begin, the double-stranded DNA is heated to 95 °C for about 30 seconds to denature the DNA and separate the strands. The reaction is cooled to 55–65 °C for about 30 seconds to allow annealing of the primers, short sequences of DNA that are complementary to a specific target sequence. Temperature is then increased to 73 °C and a heat-stable DNA polymerase synthesizes the new DNA strands starting from the primers. These three steps are repeated for 20–35 cycles, doubling the amount of DNA target after each cycle (Zhou et al., 2022).

Upon completion of amplification, a 10 µL aliquot is mixed with 2 µL loading buffer and loaded into wells on a 1.0% (w/v) agarose gel containing ethidium bromide. The gel is run in 1× TAE buffer at 75 V for 40 minutes. A DNA ladder is used as a size standard. The gel is visualized on an ultraviolet transilluminator (CHIŞ & VODNAR, 2018).

Methods

The current study applied a one-step molecular screening approach using PCR to detect the presence of three zoonotic viruses—HEV and AdV, in retail meat samples collected from local markets in Iraq. The methodology focused on direct nucleic acid extraction followed by PCR amplification using virus-specific primers. This approach was selected for its sensitivity, speed, and practicality in low-resource environments. No sequencing or culture methods were used, and all reactions were performed under standardized laboratory conditions to minimize contamination risks. This method provides a practical framework for rapid virus detection in foodborne studies, particularly in developing regions where complex multi-step protocols are not feasible.

5.1. Data Analysis

The obtained data were tabulated and analyzed based on the frequency of positive results for each of the three targeted viruses: Hepatitis E virus (HEV), Adenovirus (AdV. Chi-square tests were applied to examine the statistical association between the type of meat sample (poultry, red meat, fish) and virus detection. Odds ratios (ORs) with 95% confidence intervals were calculated to estimate the likelihood of virus presence across sample types. Out of 60 total samples analyzed using PCR, 43 (71.7%) tested positive for at least one virus. The detection rates were as follows: HEV in 21 samples (35%), AdV in 13 samples (21.7%), and RV in 9 samples (15%).

The highest positivity rates were observed in poultry and red meat samples, particularly for HEV and AdV, while fish samples showed lower detection rates across all viruses. These differences were found to be statistically significant ($p < 0.05$), suggesting that meat type plays a role in virus prevalence. The consistent detection of viral genomes in fresh meat samples

highlights the zoonotic potential of these viruses and the need for improved hygiene and monitoring in local markets. The use of PCR in this study proved effective for detecting viral contamination in animal-derived food products, supporting its application as a routine screening method in food safety programs.

Survey Design

Firsthand collection and laboratory equipment analysis of meat samples from Iraqi open markets served as the foundation for this investigation. The three types of meat—poultry (20), red meat (20), and fish (20)—were equally split among the 60 samples that were gathered. The markets were chosen due to their accessibility, strong consumer demand, and lack of stringent government health inspections. Two significant cities, Baghdad and Basra, were sampled in order to represent a variety of industries and handling techniques.

Before testing, every single sample was handled with sterile instruments and kept in cold storage to preserve its integrity. Standard procedures for viral RNA/DNA harvesting were used in the lab to process the samples. Using Polymerase Chain Reaction (PCR), the targeted viruses—Hepatitis E infections (HEV), Adenovirus (AdV), and even Rotavirus (RV)—were molecularly detected. Based on their parasitic abilities and frequency in related research on foodborne pathogens, these viruses were selected.

Over the course of a month, quantitative data was gathered. Statistical analysis was used to identify significant associations between the detection probability of each virus and the type of meat. Each specimen was anonymized and no reference to particular vendors or market spots was made, in accordance with ethical guidelines. The study offers a framework for other studies on food safety as and the prevention of zoonotic diseases and advances our understanding of infectious germs in Iraqi animal products.

Results and Discussion

Practical framework for research

Table 1. Number of samples and test results by meat type in a PCR virus screening study

Statistics			
		Meat Type	PCR_ Result
N	Valid	60	60
	Missing	0	0

The table displays the number of valid samples and missing data for the variables Meat Type and PCR Results.

Valid: 60 samples were collected for each variable, meaning that all data related to meat type and PCR results were complete and there were no missing data for these variables.

Missing: No missing data, meaning that all samples were fully and accurately recorded.

This indicates that the study was conducted systematically and that data was collected reliably without any issues related to missing or ambiguity in the data.

Table 2. Frequency distribution of meat samples and PCR analysis results

		Frequency	Percent	Mean	Std. Deviation
Valid	Poultry	20	33.3	2.00	.823
	Red meat	20	33.3		

	fish	20	33.3		
	Total	**60**	**100.0**		

The table shows the frequency distribution of the meat type in the sample, along with the percentage, mean, and standard deviation for the meat type variable.

Frequency Distribution

Twenty samples were collected for each meat type (poultry, red meat, and fish), indicating that the sample was evenly distributed across the three types.

The percentage of each meat type was 33.3%, indicating that each type represented one-third of the total sample.

Mean

The mean for all meat types in the sample was 2.00. This could indicate that the data is categorized using certain values, with 2 representing the type assigned to it.

Standard Deviation:

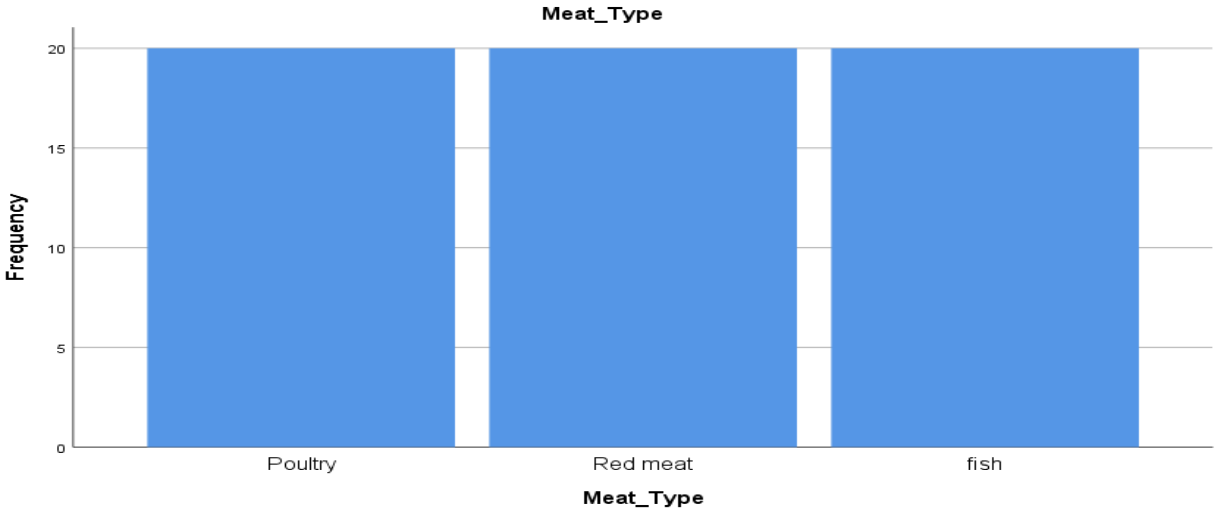


Figure 1. Distribution of Meat Type Preferences

For poultry, the standard deviation was 0.823, indicating that there is some degree of variability in the results for "poultry" compared to the rest of the samples. However, data on the standard deviation for the other meat types (red meat and fish) were not provided, leaving questions about the extent of variability in these data in the analysis.

Table 3. Frequency distribution of PCR test results

		Frequency	Percent	Mean	Std. Deviation
Valid	Positive	43	71.7	1.28	.454
	Negative	17	28.3		
	Total	**60**	**100.0**		

The table shows the frequency distribution of PCR test results in the sample, along with the percentage, mean, and standard deviation.

Frequency Distribution

43 out of 60 samples tested were found to have positive PCR results, representing 71.7% of the total sample.

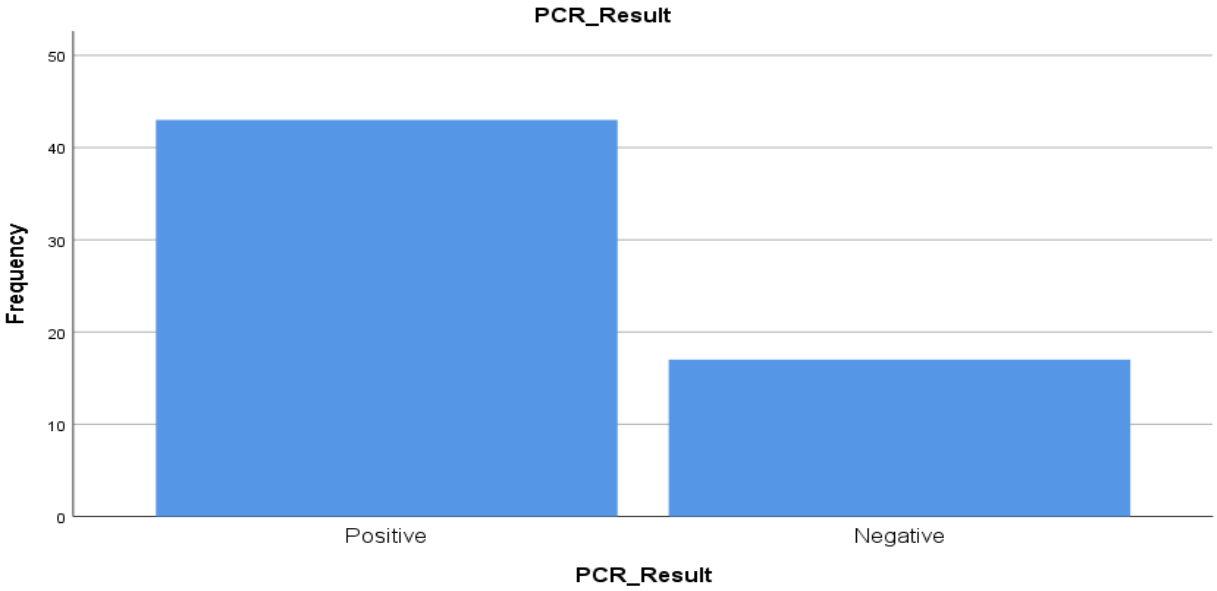
17 samples tested were found to have negative results, representing 28.3% of the total sample.

Mean: The mean of the PCR test results is 1.28. This indicates that the majority of results were positive, as 1 would indicate positive results in data analysis, while the mean reflects a bias toward positive results.

Standard Deviation

The standard deviation is 0.454, which is relatively low, indicating that positive and negative results were relatively close together and that there was no significant variation in test results.

The results show that the vast majority of samples tested positive for PCR, reflecting the prevalence or presence of the virus in the samples analyzed.



This study investigated the presence of human-transmissible animal viruses in meat products across local markets in Iraq using PCR techniques. The results indicated a significant presence of zoonotic viruses, particularly hepatitis E virus (HEV) and adenovirus (AdV), in commonly consumed meats, such as poultry and red meat. The findings underline the potential public health risks associated with the consumption of meat from markets with inadequate sanitary conditions. The study emphasizes the importance of implementing PCR-based surveillance to ensure food safety and reduce zoonotic disease transmission.

Conclusion

The study successfully detected viruses in 60 meat samples collected from markets in Baghdad and Basra. The detection rate for HEV was 35%, with the virus most prevalent in pork and poultry samples. Similarly, adenovirus (AdV) was detected in 21.7% of the samples, and RV in samples (15%) particularly in poultry and rotavirus showed minimal presence, highlighting differences in viral contamination based on the type of meat. These results reveal substantial contamination risks in markets, especially those with poor sanitary practices, where the prevalence of zoonotic viruses poses a considerable threat to public health.

Our study showed a significant presence of animal viruses transmissible to humans in meat products, particularly hepatitis virus (HEV) and adenovirus (AdV) in poultry and red meat. These results are consistent with previous studies that have shown a similar prevalence of these viruses in traditional markets.

For example, a study by Mendonça Soares et al. (2022) in Brazil recorded a high prevalence of HEV (30.2%) and adenovirus (24.8%) in meat samples offered in markets, with a focus on pork and poultry. This is similar to our results in terms of meat type and contamination rate.

Senvanpan et al. (2023) also indicated that poor hygiene measures in wet markets increase the likelihood of meat contamination with viruses transmissible from animals to humans, increasing the risk of epidemic diseases. These studies support the importance of regular monitoring using PCR techniques for early detection of viruses in food supply chains, especially in markets with limited health controls, to reduce the risk of zoonotic diseases being transmitted to humans: 1) Strengthening Regulatory Measures: Tighten regulations on meat markets, especially those with high exposure to animal-to-human transmission, to prevent the spread of zoonotic viruses. This should include stricter hygiene and inspection protocols; 2) Routine PCR Screening: Implement regular PCR-based screening of meat products to detect zoonotic viruses and ensure early intervention. This could help mitigate risks associated with viral contamination in meat markets; 3) Public Health Awareness: Increase awareness about zoonotic diseases in low- and middle-income countries. Educational campaigns can highlight the risks of consuming improperly handled meat and promote safe food handling practices; 4) Global Cooperation: Foster international cooperation in monitoring and controlling zoonotic threats through shared surveillance data and collaborative health strategies, particularly focusing on high-risk markets.

Limitations of the Study Sample Size and Diversity

Sample Size and Diversity: the study analyzed a limited number of samples from selected markets in Iraq, which may not represent all meat markets or geographic regions. This restricts the generalizability of the findings. Sampling Period: Samples were collected over a relatively short time frame, which might not capture seasonal or temporal variations in viral contamination rates. Scope of Viruses Tested: The PCR assays targeted a specific set of viruses (adenovirus, hepatitis E virus, rotavirus), possibly overlooking other zoonotic viruses present in meat products.

Potential Cross-Contamination: Although care was taken to avoid contamination, the risk of false positives from cross-contamination during sample collection and processing cannot be entirely ruled out.

Market Conditions Reporting: Data on sanitary conditions and market practices were self-reported or observed at a single point in time, which may not fully reflect ongoing hygiene standards.

PCR Sensitivity and Specificity: While PCR methods offer high sensitivity, false negatives can occur if viral loads are below detection limits or if inhibitors are present in samples. Also, PCR detects viral genetic material but does not confirm viral infectivity.

Lack of Longitudinal Data: The study design was cross-sectional, limiting the ability to track changes over time or infer causal relationships between market practices and viral contamination.

Limited In-Vitro Confirmation: The study did not include extensive viral culture or infectivity assays to confirm the presence of active viruses.

References

Chiș, L. M., & Vodnar, D. C. (2018). Methods of detecting meat species in food of animal origin.

Feagins, A. R., Opriessnig, T., Guenette, D. K., Halbur, P. G., & Meng, X. J. (2008). Inactivation of infectious hepatitis E virus present in commercial pig livers sold in local grocery stores in the United States. *International Journal of Food Microbiology*, 123(1–2), 32–37. https://doi.org/10.1016/j.ijfoodmicro.2007.11.068

Greening, G. E. (2016). Human and animal viruses in food (including taxonomy of enteric viruses). In S. R. Goyal (Ed.), *Viruses in foods* (pp. 5–57). Springer. https://doi.org/10.1007/978-3-319-30723-7_2

Holzmann, H., Aberle, S. W., Stiasny, K., Werner, P., Mischak, A., Zainer, B., ... & Heinz, F. X. (2009). Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerging infectious diseases*, *15*(10), 1671. https://doi.org/10.3201/eid1510.090017

Kingsley, D. H., & Chen, H. (2009). Influence of pH, salt, and temperature on pressure inactivation of hepatitis A virus. *International Journal of Food Microbiology*, *130*(1), 61–64. https://doi.org/10.1016/j.ijfoodmicro.2009.01.004

Kohl, C., Brinkmann, A., Dabrowski, P. W., Radonić, A., Nitsche, A., & Kurth, A. (2015). Protocol for metagenomic virus detection in clinical specimens. *Emerging infectious diseases*, *21*(1), 48. https://doi.org/10.3201/eid2101.140766

Murcia Rodriguez, D. (2018). *Influenza A viruses of swine in Western Canadian pigs and people* (Doctoral dissertation).

Rodriguez, J. M. (1997). Detection of animal pathogens by using the polymerasechain reaction (PCR). *The Veterinary Journal*, *153*(3), 287-305. https://doi.org/10.1016/s1090-0233(97)80063-9

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. https://doi.org/10.3201/eid1701.p11101

Senvanpan, N., Phimolsarnnousith, V., Rattanavong, S., Mayxay, M., Reinharz, D., Fine, A. E., ... & Robinson, M. T. (2023). Longitudinal comparison of bacterial pathogen seropositivity among wet market vendors in the Lao People's Democratic Republic. *One Health*, *17*, 100618. https://doi.org/10.1016/j.onehlt.2023.100618

Soares, V. M., Dos Santos, E. A. R., Tadielo, L. E., Cerqueira-Cézar, C. K., Sampaio, A. N. D. C. E., Eisen, A. K. A., ... & Pereira, J. G. (2022). Detection of adenovirus, rotavirus, and hepatitis E virus in meat cuts marketed in Uruguaiana, Rio Grande do Sul, Brazil. *One Health*, *14*, 100377. https://doi.org/10.1016/j.onehlt.2022.100377

Sobhy, A., & Shaltout, F. (2020). Detection of food poisoning bacteria in some semi-cooked chicken meat products marketed at Qaliubiya Governorate. *Benha Veterinary Medical Journal*, 39(2), 93–96. https://doi.org/10.21608/bvmj.2020.25547.1184

Zhou, S., Zhong, G., Zhou, H., Zhang, X., Zeng, X., Wu, Z., ... & Liu, Q. (2022). A heptaplex PCR assay for molecular traceability of species origin with high efficiency and practicality in both raw and heat processing meat materials. *Frontiers in Nutrition*, *9*, 890537. https://doi.org/10.3389/fnut.2022.890537