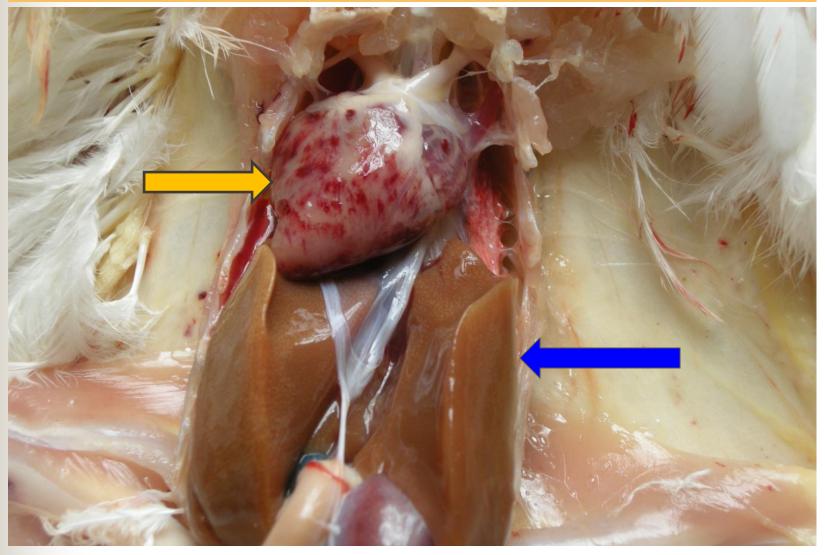


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## **Clinical Signs & Lesions**

	Age weeks	No. of flocks		Her	norrhages		Anemic appear ance	Bone marrow (pink/ yellowish)	Mortal ity %
and the second second			SC	Prov.	Int.org	Heart			
Contraction of the	1-2	8	+3	+1	+3	+2	+3	+3	12.5
AND COL	3-4	9	+4	+2	+2	+3	+3	+3	14
VALUE AV	5-6	8	+4	+2	+4	+4	+4	+4	10.8
No. Control	7-8	30	+4	+3	+4	+4	+3	+3	12
all a state	9-10	9	+3	+1	+2	+1	+3	+2	9.5





# Hematological findings and PCR results

Farm no.	Hct.	Hb	PCR result of tissues		
			Liver	Spleen	
1	11.80	5.50	-	-	
2	10.90	6.00	-	-	
3	12.25	5.25	+	-	
4	11.00	6.16	+	+	
5	11.60	6.29	+	-	
6	13.20	6.50	+	-	
7	17.20	6.70	+	+	
8	10.85	5.70	+	-	
9	12.30	6.28	+	-	
10	12.00	6.00	+	-	

# **Gross lesions**

- Liver and bone marrow pale in color appearance
- Thymus atrophied
- Mortality ranged from 5-14% on different farms
- The maximum mortality was in 5-6 week age group.
- Samples of liver and spleen from 60 farms selected on the basis of necropsy for PCR analysis
- Highly conserved VP2 coding gene using CAV1 and CAV2 primers was amplified (186-bp region) by PCR for confirmation of CIAV.
- A total of 52 (86.66%) farms out of 60 were positive for CIA infections on PCR based results





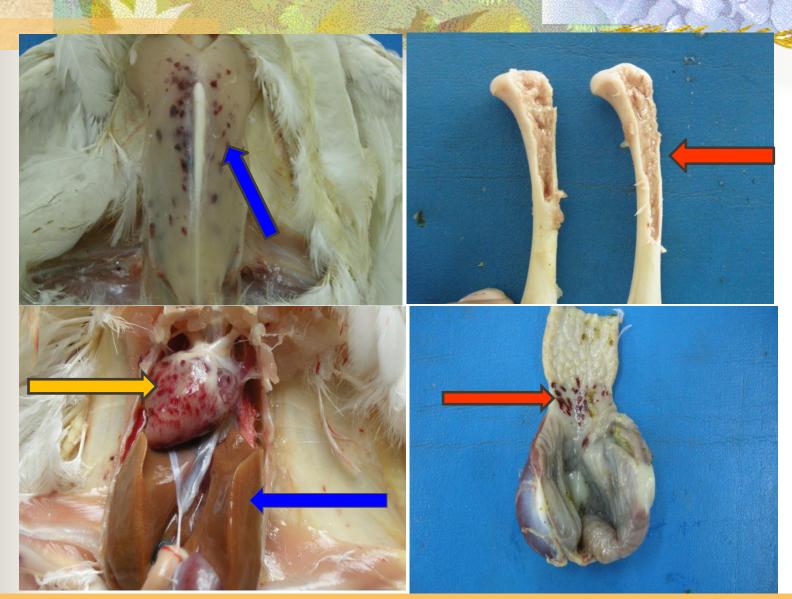


Fig. 3: Photograph of CAV infected birds indicating haemorrhages on pectoral muscles, hear, gizzard and pale/white bone marrow and anaemic liver





#### **PCR Positive Samples**



**Fig. 4.** Detection of CAV by PCR and amplification of highly conserved VP2 coding gene specific PCR product(186bp) Lane 1 Positive Control, Lane 2 Negative Control and 3-5 Positive samples





# Phylogenetic analysis of Recent Isolates (2018)

- Nucleotide Sequence and Phylogenetic analysis of 2018 recent cases was performed at HVRI, Harbin China
- All the 14 CAV VP2 sequences showed 99% nucleotide identity for VP2 region in GeneBank.
  - All the sequences in this study were closely related.
- The result showed samples and the reference sequence has common ancestor (Figure. 5)
- The authenticity of PCR amplification of VP2 was confirmed by the nucleotide sequencing.





#### **RESULTS: Phylogenetic Analysis**

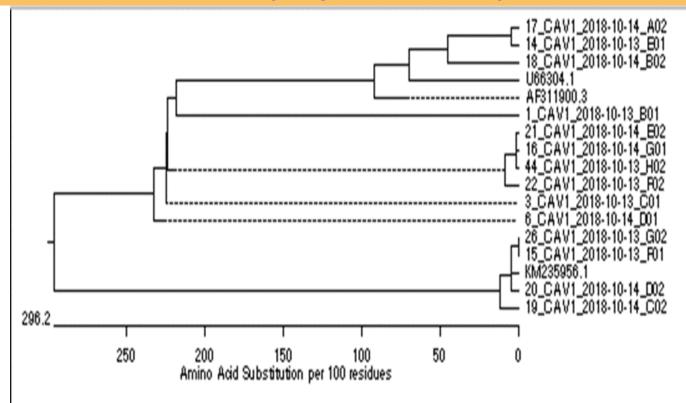


Fig. 5: Phylogenetic relationship among 14 different CAV isolates based on partial nucleotide sequences



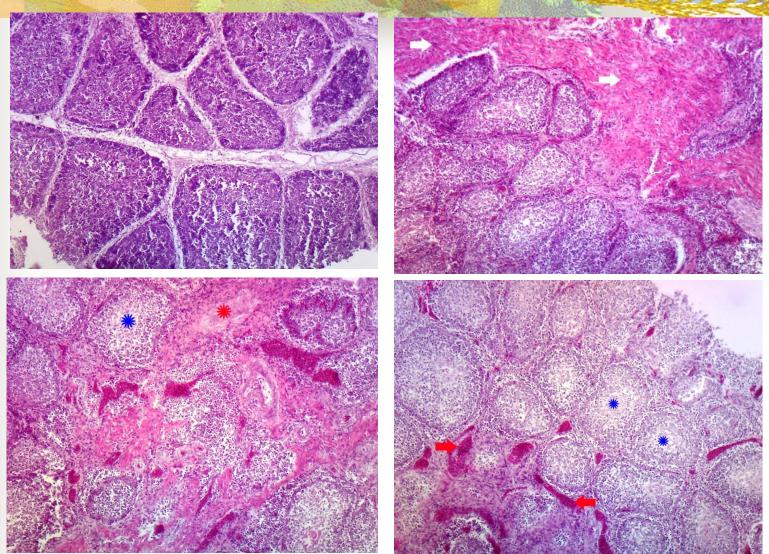


#### **Nucleotide Sequence and Phylogenetic analysis**

- All the 14 CAV VP2 sequences showed 99% nucleotide identity for VP2 region in Genebank.
  - All the sequences in this study were closely related.
- The result showed samples and the reference sequence has common ancestor (Figure 3).
- In Fig.3 sample no. 14, 17 & 18 were closely related (99%) to United kingdom (U66304) and Alabama (AF311900), USA. While sample no. 15,19,20 and 26 were closely related (99%) to Japan (KM235956).

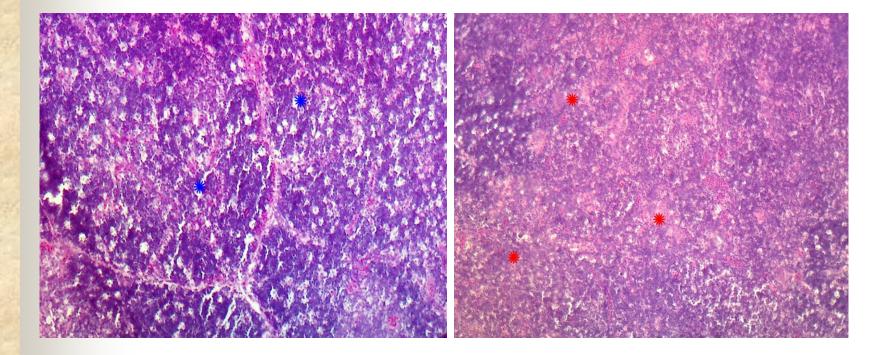






**Fig. 6:** Photomicrograph of control group (a) in CAV group increased interfollicular connective tissue (b & C) and lymphocytic depletion in (c & d) (H&E Staining 100X)





**Fig. 7:** Photomicrograph of thymus from CAV infected birds showing lymphocytic depletion(a) and necrotic changes(b) in the parenchyma (H& E Staining 100X)





First Outbreak Report from Pakistan by Department of Pathology, UAF (Najam-ul-Islam et al., 2013)



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RESEARCH ARTICLE

Molecular Diagnosis and Pathology of Chicken Infectious Anemia in Commercial White Leghorn Layer Flocks in Pakistan

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## Data of 2013-14 Case Study: Diag. Lab data

- Total 86 Farms samples were collected from layer chicks during rearing period from Faisalabad division and Sargodha
- More than 80% positive for CAV infection
- Cases of 5-18 week age groups were also involved
- Severe immunosupression poor response towards other vaccine like ND, IB, IBD, AI etc.
- In 2012-13 Disease situation was comfortable and few cases were diagnosed





#### Data of 2016-17 Cases

- Total 57 farms samples were submitted to diagnostic laboratory from Faisalabad and surrounding areas and Sargodha
- The 41 (72%) farms out of these 57 were positive for CAV infection through PCR
- Cases of 4-16 week age groups were recorded
- All the above mentioned classical signs and lesions were observed in these birds
- In 2015-16 situation was quiet comfortable





## Data of 2017-18 Cases

- In this year layer in rearing about 84 farms samples were collected
- Tissues samples (220) from 44 farms were processed through PCR
- Out of these 44 farms 28 (63%) farms were positive for CAV
  - All the classical sings of CAV were observed in the birds
- Mortality is observed all age groups





# Data of 2018-19 Cases

- Total no of samples: 765 Samples from 153 farms
- 95 samples out of total 765 were found positive for CIA through PCR
- Therefore 12.41 % samples were positive for CIA
- Distribution of samples among Layers and Broilers
- Layer: 285 Samples (60 samples 21.05%)
- Broilers: 460 Samples (25 samples 5.43 %)
- Broiler Breeders: 10 Samples (0 % )
- Layer Breeder: 5 samples (5 samples 100 %)
- Non-Descript Bird: 5 Samples ( 5 samples 100 %)





#### **CAV in Broilers** (Saif et al., 2018) Department of Pathology UAF

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#### Full Length Article

#### Molecular Epidemiology and Pathology of Chicken Infectious Anemia in Day Old Broiler Chicks in Faisalabad, Pakistan

Saif-Ur-Rehman<sup>1</sup>, Muhammad Kashif Saleemi<sup>1</sup>, Muhammad Zargham Khan<sup>1</sup>, Ahrar Khan<sup>1</sup>, Asim Shahzad<sup>1</sup>, Aisha Khatoon<sup>1</sup>, Ahad Fayyaz<sup>1</sup>, Bilal Aslam<sup>2</sup>, Muhammad Sohail Sajid<sup>3</sup>, Masood Akhtar<sup>4</sup> and Rao Zahid Abbas<sup>3</sup> <sup>1</sup>Department of Pathology, University of Agriculture, Faisalabad-38040, Pakistan <sup>2</sup>Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad-38040, Pakistan <sup>3</sup>Department of Parasitology, University of Agriculture Faisalabad-38040, Pakistan <sup>4</sup>Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan, Pakistan \*For correspondence: drkashif313@gmail.com

# Total 254 samples analyzed 38/254: 14.96 % Positive for CIA through PCR





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# AS-74 (2017-2020)

"Pathobiology and Molecular Epidemiology of Chicken Infectious Anemia and Infectious Bronchitis (IB) in Commercial Poultry and Immunopathogenesis of IB Variants"

• Avian & Toxicologic Pathology Laboratory Team, UAF