

## Preparation of Frozen Bivalent Babesial (*Babesia Bovis* and *Babesia Bigemina*) Vaccine from Field Isolates and Evaluation of Its Efficacy in Calves

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**Abstract :** Babesiosis is reflected as the most important disease of cattle that are transmitted by arthropods. In Pakistan, its prevalence is up to 29% in the cattle and buffalo population in different regions. Cattle show a long lasting and durable immunity by giving an infection of *B.bovis*, *B. bigemina*, or *Babesiadivergens*. this is used in cattle to immunize them in a few countries as anti-babesiosis vaccine. Development of frozen vaccine allows for complete testing after production of each batch, However, once thawed, its reduced its shelf life, frozen vaccines are more difficult to transport as well as expensive to produce as compared to chilled vaccine. The contamination of blood derived vaccine has the potential risk that makes pre-production and post-production quality control necessary. For the trail master seed production of whole blood frozen bivalent *Babesia*(*Babesiabovis* and *Babesiabigemina*), 100 blood samples of Babesial positive suspected cattle was taken and processed for separation microscopic detection and rectification by PCR. Vaccine passages were done to reduce the parasitaemiasis in live calves. After 8 passages, parasitemia of *Babesia* reduced from 80% to 15%. Infected donor calf's blood was taken by jugular cannulation by using preservative free lithium heparin as an anticoagulant (5 International Units IU heparin/ml blood). In lab, parasite containing blood was mixed in equal volumes with 3 M glycerol in PBS supplemented with 5 mM glucose (final concentration of glycerol 1.5 M) at 37°C. The mixture was then equilibrated at 37°C for 30 minutes and were dispensed in required containers (e.g., 5 ml cryovials).

**Keywords :** distribution, babesia, primer sequences, PCV

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